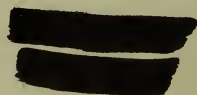




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BUREAU OF ANIMAL INDUSTRY.—BULLETIN 137.

A. D. MELVIN, CHIEF OF BUREAU.

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# ANTHRAX,

WITH SPECIAL REFERENCE TO THE PRODUCTION  
OF IMMUNITY.

BY

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Bureau of Animal Industry.*



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1911.

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## LETTER OF TRANSMITTAL.

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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF ANIMAL INDUSTRY,  
*Washington, D. C., March 20, 1911.*

SIR: I have the honor to transmit for publication the accompanying manuscript on the subject of anthrax, by Dr. Charles F. Dawson, of the Delaware College Agricultural Experiment Station, the experimental work having been carried out in cooperation with the Pathological Division of this bureau.

Anthrax is one of the oldest and most widely distributed diseases of animals, and is particularly destructive to cattle and sheep. The existing method of combating the infection is to endeavor to control it by means of a vaccine devised by Pasteur. Two injections are, however, necessary by this method, and an active immunity is not established for about four weeks.

Two important results have been accomplished through the work described in this paper. A single vaccine has been produced which may be substituted for the above-mentioned double vaccine, thus cutting down the time one-half. The other result is the production of an antianthrax serum that will confer an immediate passive immunity. Thus the two combined are a distinct advantage over the existing method in the case of sudden outbreaks, where there is no time to prepare for the disease in the usual way. This serum can also be used in conjunction with the double vaccine of Pasteur.

I recommend that the paper be published in the bulletin series of this bureau.

Respectfully,

A. D. MELVIN,  
*Chief of Bureau.*

Hon. JAMES WILSON,  
*Secretary of Agriculture.*



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# ANTHRAX,

WITH SPECIAL REFERENCE TO THE PRODUCTION OF IMMUNITY.

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## INTRODUCTION.

Anthrax is primarily a disease of herbivorous animals, and occurs as an epizootic in sheep, cattle, horses, and mules, the animals being susceptible in the order named. In experimental work the small animals, such as mice, guinea pigs, and rabbits, are extremely susceptible. The French name for the disease is "charbon;" the German, "Milzbrand." When it occurs in domestic animals it is also sometimes known as splenic fever. In natural outbreaks anthrax usually has the character of a rapidly fatal septicemia, with the presence of large numbers of the characteristic bacilli in the blood.

While the disease does not occur as a natural infection in man in the same sense as in animals, man frequently becomes infected from handling infected animals or their products, such as hides and hair. When man becomes infected through abrasions of the skin, the resulting disease process is carbunculous in nature and is known as "malignant pustule." When the infection takes place in the lungs, the disease is known under the name "woolsorters' disease." A third form in man may attack the intestinal tract. The latter two forms are rapidly fatal. In malignant pustule the disease may remain localized and the patient may recover. If, however, the bacilli migrate from the local lesion to the blood stream, death rapidly ensues, the patient dying from septicemia or toxemia.

## HISTORICAL.

Anthrax is one of the oldest diseases. Moses records it in Exodus 9:9. Homer, Ovid, Plutarch, Virgil, Pliny, and many others have mentioned it in their writing. It exists in all countries and in all latitudes. It was formerly very destructive to human life as well as to animals. In 1617, near Naples, 60,000 people are reported to have died from anthrax. In San Domingo, in 1770, 15,000 persons perished in the short period of six weeks. This enormous death rate was probably due, in part, to eating the carcasses of animals dead from the disease.

It was not until the middle of the nineteenth century that scientists began the serious study of this very destructive disease. In 1849, Pollender, while studying the blood of animals dead from anthrax, discovered numerous rod-shaped, microscopic bodies, which he claimed were the cause of the disease. This claim remained unsubstantiated until 1863, when Davaine announced that the bodies discovered by Pollender were bacteria, and showed that the blood of an animal could not cause anthrax in another animal when injected into it unless it contained these bodies. To this bacterium Davaine gave the present name, *Bacillus anthracis*. Davaine's views were not immediately accepted, but others took up the work, and in 1876 Dr. Robert Koch, in making his first contribution to bacteriology, announced that he had been successful in proving the correctness of Davaine's work on anthrax, and the question was then accepted as settled. Koch isolated the bacilli in pure culture and demonstrated their ability to form spores. He claimed that natural infection takes place through the intestinal tract, although he was at first unable to demonstrate this by feeding food infected by artificial cultures. Later, Pasteur succeeded in producing anthrax by feeding hay sprayed with cultures of *Bacillus anthracis* when he at the same time mixed with the hay thistles or any plants that would cause pricking or abrasions of the intestinal tract so that the bacilli could pass into the tissues.

#### THE ANTHRAX BACILLUS.

On account of the comparatively large size of the *Bacillus anthracis*—it being from 5 to 20 micromillimeters long and from 1 to 1.5 micromillimeters broad, although both longer and shorter forms occur—and the great readiness and certainty with which it kills experimental animals, it was the earliest disease to be studied with the microscope and by modern bacteriological methods. We first learned to demonstrate spore formation and spore staining from anthrax cultures. The germ is exceedingly easy to cultivate, growing on almost any medium and at a wide range of temperature. It has many of the general morphological characters of the whole group of bacteria, and is, therefore, very useful for teaching purposes. The bacillus has a place in the earliest researches on immunity, Pasteur having shown in 1880–1882 that animals inoculated with strains of the bacilli which had been devitalized to a certain extent by cultivation at high temperatures did not contract anthrax when exposed, while those not so protected did contract the disease. Spores of anthrax are among the most resistant things in nature, and are, therefore, useful in testing the germicidal power of disinfectants.

If a drop of blood is taken immediately after death from an animal dead from anthrax and examined with a microscope, large numbers

of nonmotile bacteria will be seen. A much better view of them can be obtained if a so-called blood-smear preparation is stained with methylene blue or any of the basic anilin dyes. It will be noticed that the ends of all the bacilli are sharply cut across. If viewed with a higher power, it will be seen that the ends bulge at the corners, making the ends appear slightly concave. The cells are also noticed to have a capsule, and when several cells are lying end to end the capsule seems common to the entire chain of cells.

If in making preparations of anthrax blood great care is taken not to overheat, but just enough to "set" the specimen, and methylene blue is used as the stain, we can, while viewing the slide by reflected light with the unaided eye, notice a violet coloration of the entire specimen. If we now place the slide under the microscope, we can see that the space between the bacillus and its capsule is stained a reddish color. This is known as McFadyeans color test, and if the specimen is properly prepared is to be observed in all anthrax preparations made from dead animals. The test is most successful in those preparations where the bacilli are very numerous, and could be of great importance in field work where a microscope may not be at hand.

#### CULTURAL AND MORPHOLOGICAL CHARACTERS.

The bacillus of anthrax has such definite and constant cultural and morphological characters that it can hardly be mistaken for any other bacterium. When growing on the surface of agar-agar plate cultures beautiful colonies occur, appearing as wavy wreaths resembling locks of hair or bits of raw cotton thrown on the surface. It is supposed that the whole mass consists of thousands of contiguous cells forming one long chain, wound up in all directions.

The growth in gelatin tubes is characteristic in that it liquefies the medium and the growth resembles an inverted fir tree.

In milk a small amount of acid is formed, so that milk is slightly coagulated and slowly peptonized.

In peptone bouillon virulent bacilli make a much more luxuriant growth than do the attenuated bacilli or vaccines. The heaviest growth is near the surface, where it can obtain oxygen, and consists of a dense mass of intertwined bacilli in chains which readily fall to the bottom of the tube if it be even slightly shaken. This heavy growth does not occur when bouillon is inoculated with attenuated bacilli.

Growth on all media proceeds best at 35° C. The minimal temperature for growth is 12° C., and the maximal 45° C. The vegetating bacillus is killed by a short exposure (15 minutes) to 60° C. moist heat, and by desiccation in a few days at ordinary temperatures. The spores, on the other hand, will stand almost any disinfecting process



that any infected material can stand without greatly changing its physical characters. Hence it is impossible to disinfect hides containing anthrax spores without spoiling them for leather making. Spores require a boiling temperature in moist heat, and a much higher temperature in dry heat to destroy them. Spores live for a long time—probably 10 years—in the soil or in water; and it is because of this great resistance that pastures once infected remain so for an indefinite period.

The bacillus can grow either in the presence or absence of oxygen, but it grows best when air or oxygen is present. Spores can form only in the presence of oxygen, and this accounts for the fact that the bacillus never sporulates in the living blood nor in cultures grown under anaerobic conditions.

When spores begin to form, a tiny bright spot appears in the center of the parent cell, which soon increases to the diameter of the cell and always remains in its center. The cell protoplasm seems to be used up in the production of the spore, which now forms the resting stage of the organism. This spore remains quiescent until it is again placed in a suitable environment for growth, when it sprouts and assumes the bacillary or infective stage.

Although the spore, or resting stage, of *Bacillus anthracis* is very resistant to all natural and artificial disinfectants, the bacillar or infective stage is quite vulnerable. The bacilli locked up in the airless blood vessels or cavities of the body not only do not spore, but rapidly disintegrate after death. These important facts are of incalculable value in the suppression of the disease by nature, and furnish the strongest kind of an argument for the prompt burial of animals dead from the disease. If such an animal be opened, however, the above-mentioned natural protection against the spread of the infection is removed, as the bacilli will continue to multiply and sporulation will finally ensue.

The following experiments were made to demonstrate the foregoing facts: The spleen of a guinea pig just dead from anthrax was minced and placed in a Petri dish exposed to air at room temperature. Microscopic examinations of this material 16 hours after showed that every bacillus had spored, the protoplasmic cellular material staining poorly. Another experiment made to determine the effect of such exclusion of the air as burial can afford showed that 24 hours are required for spore formation.

In order to determine the effect of total exclusion of air upon the question of viability of the bacilli, capillary glass tubes were filled with heart's blood from a mouse just dead from anthrax and sealed in a flame. These sterile tubes were filled under precautions which prevented the ingress of extraneous bacteria; hence there were only anthrax bacteria in them, and the disintegrating effects of post-



mortem bacilli through the septic ferments which they form in a decomposing carcass were not present. Examinations and cultures from these tubes were made at intervals up to the twenty-third day, and while the bacilli were lessened in numbers, they were still viable. In buried carcasses, even though they be not opened, putrefactive bacteria grow very rapidly and their excretions cause a granular degeneration of the anthrax bacilli. If air is admitted to the carcass these excretions have no deleterious effect upon the spores that have formed, and such a carcass infects the soil in which it is buried, while if the carcass had not been opened, and care had been taken to disinfect the soiled exterior and air-containing cavities of the animal, the putrefactive bacteria would kill off the anthrax bacilli. The length of time necessary for this destruction varies with the temperature, being about a week in hot weather.

#### CHANNELS OF INFECTION.

There are four routes by which animals may become infected, namely, by ingestion, by inoculation, by inhalation, and by transmission.

*Ingestion.*—The first and most frequent, and therefore the most important, is by ingestion. This form is acquired when animals are turned out to pasture.

*Inoculation.*—The method of infection next in importance is by inoculation, which is brought about by contact of abrasions and wounds with infected material, or by flies or other insects.

A case of spontaneous inoculation arising in the course of my experiments—the only one which occurred in the work—was where a ram, immune to anthrax, was severely bled to furnish blood for experiments. He was kept in a stall where a sheep had died three months previously of experimental anthrax. A considerable wound was made by the operation and the animal was found dead of anthrax on the second day after having been bled.

*Inhalation.*—The form third in importance is by inhalation. Here spores are breathed in with dust arising from infected material or surfaces.

*Transmission.*—A fourth form, which is probably rare, is that of transmission from the mother to the fetus in utero. I have observed this in the case of an experimental cow which had been immunized with a single anthrax vaccine and subsequently tested with virulent bacilli. The mother at no time appeared sick, but on the sixteenth day after receiving the virulent bacilli she dropped a dead calf, which appeared to be a full-term one. Not suspecting the cause of the trouble, the calf and membranes were buried. Fortunately, a loop of the discharge found on the ground was plated and developed a pure cultivation of 60,000 anthrax bacilli, the virulence of which

was demonstrated on a guinea pig. The cow was in no way sick, and daily microscopic examinations of the vaginal discharges showed these were free of anthrax bacilli.

### INFECTION OF THE SOIL.

Soil becomes infected with anthrax by discharges from animals suffering with the disease, and by the dead bodies of animals which are allowed to decompose upon the surface. These discharges consist of small hemorrhages which occur from the mucous membranes in the nose and bowels, mixed with the nasal secretions and with the feces. These sometimes do not occur until just before death. In some cases the discharges are so limited as almost to escape observation, but a microscopic examination in such cases would reveal large numbers of bacilli. Hence it is very important to limit to the smallest area possible the wanderings of an animal so affected. It is doubtful if the feces of the smaller experimental animals contain anthrax bacilli. One observer reports the presence of anthrax spores in the feces of guinea pigs, but I have never found them.

Pasteur, in his memorable paper on anthrax before the Academy of Sciences in Paris in 1880, gave his opinion as to another manner in which the surface becomes infected with this disease. He said in part:

Earthworms are the bearers of the germs, and they bring this terrible parasite to the surface of the ground from the depths to which it has been buried. It is in the little cylinders of earth, of fine earthy particles, that the worms excrete and deposit at the surface of the ground after morning dews, or after rains, that are to be found, besides a crowd of other germs, the germs of charbon (anthrax).

While it does not seem impossible that earthworms may swallow the spores of anthrax and cast them out upon the surface, that close observer, Koch, was unable to verify the facts. He held that pastures remain infected on the surface from preexisting cases, and that the parasite remains on or near the surface from season to season and infects animals which graze upon such soil. When the grass gets short in dry weather, spores that may have been carried into the soil by previous rains either rise again by capillarity or are brought to the surface by the animals pulling the grass up by the roots. The writer has examined many earthworm casts from anthrax graves of various ages—from 15 years to 1 month—and has failed to find anthrax spores in them. Pasteur's work was done before the days of solid media, and hence the plate-culture method of isolation was not used by him. Another disease, malignant edema, is easily produced in guinea pigs and rabbits, and greatly resembles anthrax clinically, as well as in the morphology of the organism

causing it. These two germs would greatly resemble each other when viewed with a microscope made in 1880.

In order to test further the question as to whether the surface above an anthrax grave becomes infected by earthworms, or otherwise, the writer has for the past three years pastured sheep and cattle upon soil in which there are many anthrax graves, and no case of spontaneous anthrax has arisen. The animals were frequently observed grazing in the little hollows formed by the sinking in of the graves, and it would seem that this is sufficient proof of the questionableness of Pasteur's early theory regarding this source of infection.

#### METHODS OF DIAGNOSIS.

Anthrax is a disease that we absolutely know is caused by the parasite ascribed to it. We can tell just how long an animal will live after it has been inoculated with a given culture, provided the culture is of maximum strength. We know that a virulent culture will, in proper dose kill the laboratory animals, as well as sheep, in 48 hours, and that we can always recover the germ in the blood of such animals and reproduce the same disease in another animal by inoculating it with this blood. Hence, all of Koch's postulates can be fulfilled.

Different races of anthrax bacilli vary in virulence. Those of highest virulence will kill guinea pigs and rabbits in 0.00001 c. c. doses of a 24-hour bouillon culture in 48 hours, and the same culture would kill a sheep in 95 hours when given a 0.0001 c. c. dose subcutaneously. Very young animals are much more susceptible to anthrax than adults, and hence care must be exercised in vaccinating young animals. A sucking rabbit a few days old died in 18 hours from a 0.1 c. c. dose of bouillon culture, and the virulence of the bacillus, which had become somewhat lessened, was greatly enhanced.

A diagnosis can be made by the veterinary scientist by two methods; either by a bacterioscopic examination of blood smears made soon after death or by animal inoculations. On account of the nature of the disease it is highly desirable that the first method be used, as no time should be lost in coming to a decision. It is easy to reach a decision if the specimen has been properly prepared and if the microscopist is familiar with the organism.

#### PREPARATION OF SPECIMENS.

A small drop of blood thinly spread on a piece of glass or paper and dried immediately is all that is required. It is best to obtain blood for this purpose by making a needle puncture into the jugular vein, as sometimes the bacilli are not numerous in the peripheral blood vessels. A specimen prepared in this way will arrive at the laboratory just in the condition in which it was collected. There will be no bacteria in it that were not there when it was prepared.



Sometimes specimens arrive consisting of two smeared surfaces of glass stuck together. These are dangerous, and it is next to impossible to separate them. They should be boiled and thrown away, no attempt being made to make an examination.

The most dangerous anthrax package, and one which is not allowed in the mails, is the bottle of blood. These have arrived broken, and in one instance a positive diagnosis was made from blood smeared on the outside of the package by the sender's hands. Again, when parts of organs, or blood in volume, are sent in, decomposition will have taken place and a diagnosis may be impossible. The fruit-jar package, containing spleen, liver, heart, etc., sent by express, is another most dangerous package. These have arrived broken, with liquid dripping from them. When not broken their opening is generally attended with dangerous difficulties. The top is usually screwed down so tight that considerable force must be used to open it, and when this is accomplished the gases which have formed will spray the liquids upon one's hands and clothing.

A diagnosis of a blood-smear preparation is made by first "setting" the specimen by heat, passing the glass slide through the flame three times, occupying a second each time. The specimen is now stained with methylene blue or fuchsin, dried between two pieces of filter paper, and mounted for examination. If anthrax bacilli are present they can be easily seen stained blue or red, according to the stain used. They appear as rods with square ends. They are never round or blunt. They will almost always appear in chains, although they frequently appear as single cells when torn apart in making the preparation. Spores are never found in a specimen prepared from a recently dead animal. With lenses of 1,000–1,200 amplification the ends appear somewhat concave, from bulging of the corners of the cylinder. When such an organism is found in the blood of an animal recently dead the disease may be called anthrax, as it is the only one in animals that provides such a picture.

#### ANIMAL INOCULATIONS.

When there is reason for doubt, animal inoculation must be resorted to. Mice, guinea pigs, and rabbits may be used, and these are susceptible in the order named. The suspected material is ground up in a sterile mortar containing normal salt solution. The solid particles are allowed to settle or are filtered off, and a few drops to 0.5 c. c. of the liquid are injected subcutaneously into guinea pigs. If anthrax be present the animal will die in two or three days, and the bacillus can be recovered from its blood and examined as in the first method. Plate cultures should also be made from the extract



of the suspected substance, as it is possible to isolate the anthrax organism and thus come to a decision from a study of the anthrax colonies if any develop, as such colonies have a characteristic appearance. Inoculation into experimental animals of cultures made from these colonies, if they be anthrax, will cause the disease, and thus any doubt as to their identity will be removed.

#### DIFFERENT FORMS OF ANTHRAX.

Anthrax manifests itself in different forms according to the seat of invasion, kind of animal, virulence of the bacillus, season of the year, and time of the outbreak.

The most acute type of the disease is seen oftenest in cattle and sheep and is known as apoplectic or fulminant anthrax. This form appears suddenly, without premonitory symptoms. The animal is suddenly seized with trembling, a haggard expression, swaying, difficult breathing, and cyanosis. Convulsions and death occur in from a few minutes to two or three hours. Bloody discharges usually occur during the last hour from the nose and rectum. These are very infectious, as the bacilli are present in large numbers, and constitute an important way in which pastures become infected. For this reason such animals should be confined where these discharges can be disinfected.

The second form, known as anthrax fever or internal anthrax, differs from the fulminant or apoplectic form only in its duration. The symptoms in the last stages are practically those seen in the most acute type, except that they are more intense and of longer duration. There will be excitability, restlessness, high fever, oozing of blood from the nose, eyes, ears, rectum, and thinner parts of the skin of the axilla or thigh; tremors, dullness, prostration, grinding of the teeth, colicky pains, difficult breathing, arching of the back, rolling of the eyes, convulsions, and death in sheep in 24 hours, in cattle in from 2 to 5 days, and in horses in from 1 to 5 days.

The third form is local or external anthrax. In this form the disease is at first localized in the mouth, throat, or skin in cattle, and the same form appears most often in horses in the tongue, throat, neck, breast, withers, shoulders, flank, or thigh. These swellings are firm or doughy, insensitive at certain parts, and tend to become gangrenous. There is no tendency to suppuration, and they do not crackle on pressure—a diagnostic point when there is doubt as to whether the case is one of anthrax or of blackleg. When cut into—something which should never be done, as the exudation always contains the anthrax bacillus—there escapes either a pale, straw-colored, or bloody liquid.

## SYMPTOMS.

The symptoms of anthrax will vary with the species and with the type of the disease, except that in the last stages of any of the three types the most pronounced symptoms are identical. In the most acute type the animal may appear at first to be perfectly well and keep along with its fellows even when its temperature is very high—as high as 106° F. Along with such a temperature we shall of course find a rapid pulse and increased respiration. When one is standing close beside such an animal the heart beats may be plainly heard. Soon other symptoms, such as grinding of the teeth, tremors, and standing with head down, appear. Then appear drooping of the head and ears and a disposition to lie down. Animals that have been lively will now decline to rise unless handled roughly. They become stupid and sleepy and very weak in the hind parts. Whereas the temperature has all along been high, it now shows a sharp decline, and before death may become subnormal. The visible mucous membranes are a dusky red, especially those of the rectum and vulva. There is a bloody nasal discharge. The feces will be coated with a bloody mucus. Local swellings appear in the mouth, throat, neck, and breast (especially in horses), and there are sharp attacks of colic and convulsions which end the misery of the animal in from 12 to 48 hours after the disease is first noticed. Pregnant animals are liable to abort and thus greatly spread the infection through the copious discharges. An outbreak has its highest mortality at its onset, while later on some animals, especially horses and mules, may recover. In the most acute types, which occur mostly in cattle and sheep, the animal is found dead. A cow which seemed well at night is found dead in the morning, or if death occurs in the daytime, the illness is of short duration, occupying only a few minutes or one or two hours. In these sudden attacks the symptoms follow each other so rapidly and death is so sudden that sometimes the owner is convinced that the animal has been poisoned. The attack is ushered in with trembling, anxious expression, high fever, rolling of the eyes, and convulsive movements, soon followed by general convulsions and death.

In the local form or cutaneous anthrax in cattle, swellings appear suddenly on different parts of the body at one or many places, and the animal dies with the same symptoms as occur in the most acute type when the bacilli reach the circulating blood from these local lesions. When the infection occurs in the tongue or pharynx, we have in the first case gloss anthrax, and in the latter pharyngeal anthrax, the symptoms varying somewhat according to the part most affected; but the general constitutional symptoms will be those already described. In some cases the most prominent symptoms at first will be enormous swellings of the rectal mucous membrane.

In the local form, or cutaneous anthrax, in the horse, the swellings occur at the points of entrance of the bacilli or spores, where there are abrasions of the skin or mucous membrane, or where biting insects have brought the infection from a previous case. These swellings appear suddenly at the point of inoculation and are characterized by a rapidly spreading edema. The general symptoms are not so urgent, the fever is less intense, and the mortality, while not so great as in the more acute form, is still high.

#### POST-MORTEM APPEARANCES.

An animal that has died of anthrax will nearly always be found much bloated, with blood oozing from the nose and rectum. There will be evidences on the ground that the animal died a violent death, in convulsions. Local swellings will be present or absent according to the type of the disease. In the rapidly fatal cases little change will be noted either in the blood or internal organs beyond those produced by high blood pressure, indicated by a swollen spleen and engorged liver. The carcass itself—the edible portions—would show nothing that would make it doubtful as food. In the more prolonged cases a hemorrhagic condition will be noted in all the internal organs. The blood will be tarry in appearance and will not clot. The heart is often light in color, while on the inside it will be found deeply stained and containing dark, uncoagulated blood. The liver may be found enlarged and is easily torn in handling, presenting on its surface hemorrhagic areas. The spleen is often specially enlarged and distorted in shape, and ruptures on handling.

The bacilli of anthrax can be found in largest numbers in those organs where the lesions are most pronounced, namely, the spleen, liver, and engorged lymphatic glands, but they are commonly found in any part of the vascular system. In the serous cavities, such as the pericardial, the pleural, and the peritoneal, may be found a sanguineous fluid consisting of serum, red and white cells, and anthrax bacilli. The hyperemia and areas of extravasated blood may appear at any point of the body where the bacilli have become localized and enormously multiplied, forming capillary embolisms, which consist of broken-down blood corpuscles, and bacilli. Hence they are frequent in the tongue, throat, lungs, stomach, and intestinal walls; the mesentery and the omentum; the skin, connective tissue, and the muscles.

#### THE CAUSE OF DEATH.

From the manner of death it would seem that the poison of anthrax acts specifically upon the center of respiration, this in turn allowing a fatal accumulation of carbon dioxid in the blood. That



it is the replacement of the oxygen of the blood by carbon dioxid that is the immediate cause of death in anthrax is further shown by the loss in the blood of the property of coagulation, it being known that carbon dioxid precipitates and throws out of action the fibrin-forming element of the blood—fibrinogen.

#### DISPOSAL OF CARCASSES.

The disposal of an anthrax carcass should not be left to the owner nor should anyone be allowed to open a carcass of an animal that is supposed to have died from anthrax. The diagnosis can only with certainty be made by a competent bacteriologist. He need only be furnished a drop of blood from the suspected animal. This blood can be drawn from the jugular by means of a syringe, or a small nick sufficient to allow a drop to escape may be made with a suitable instrument. This blood should be smeared upon a piece of glass or piece of paper, dried immediately, and forwarded to the bacteriologist. Any blood escaping during the above operation should at once be disinfected. The State should appoint a sanitary agent in every election district, whose duty it should be to dispose of anthrax carcasses, according to instructions issued by a competent authority, as it is only by the proper disposal of the carcass and by vaccination that the disease can be controlled. While the majority of our farmers recognize the advisability of promptly burying the carcass, there are those who would allow the animals to lie where they die to decompose or be eaten by dogs and buzzards. Parts of the carcass may be carried by dogs to adjoining or distant pastures and spread the infection. Spores forming in the carcass opened in this way would pass through the intestinal canal of buzzards and dogs and permanently infect pastures miles away. The soil upon which such an animal has lain would certainly be infected and remain so for many years.

During the last few years deep burial, rather than efforts at cremation, has been practiced, with good results so far as can be ascertained. While cremation properly carried out is undoubtedly the safest method of disposing of an anthrax carcass, it is practically impossible to destroy absolutely all vestiges of the body and bacilli-laden body fluids, and unless this is done the whole operation so far as it goes is almost a useless waste of time, material, and labor. In the cremation of carcasses in the field even the earth upon which it has lain should be thoroughly and deeply burned over so that the heat will penetrate to a depth sufficient to insure the death of the anthrax bacilli which certainly passed into the soil with the body fluids when rupture occurred from the heat. We know enough of the biology of the anthrax bacillus to take advantage of the fact that if it is kept locked up in the closed cavities and blood vessels of the body where no air can reach it, not only will the bacilli fail to produce the re-



sistant spore, but they will actually decompose and become noninfectious, so that the bacilli which are in the blood vessels of an unopened animal perish. On the other hand, those bacilli which have come away in the pulmonary and rectal discharges during life will, if they are provided with sufficient material to keep them moist, promptly go into the spore or resting stage. These constitute the main source of danger, and in this way a considerable area may become infected. For similar reasons, hemorrhages into air-containing cavities, such as the lungs, trachea, head, sheath, vagina, and rectum, all favor the production of the resistant spore. Hence, if we introduce into the cavities a disinfectant which will surely kill the spores, and also at the same time take care to disinfect the soiled exterior surfaces of the animal, and then bury such an animal without opening it, we may reasonably conclude that such a carcass will not thereafter be a source of infection.

#### THE OFFICIAL METHOD IN DELAWARE.

In Delaware the method of procedure as advised and as carried out by the State board of agriculture is as follows: Formalin is injected in both directions into the trachea. Formalin-soaked cotton is pressed up the nostrils and into the mouth, rectum, vagina, or sheath. The grave is dug where the animal dies, when possible. The body of the animal is then either thoroughly wetted down with a 4 per cent solution of formalin or kerosene, or coal oil is poured over the uppermost side of the animal and is set on fire. Only enough kerosene is applied to burn off the hair, as it would defeat the purpose to burn the animal sufficiently to cause rupture of the body. The carcass is then rolled into the grave with the other side uppermost. This side is now sprinkled with the oil and fired, after which the dirt upon which the animal has lain is disinfected with formalin and is then shoveled into the grave first. The grave is now filled and banked up as is the custom. Such graves should be surrounded by a fence. In fact, there should be a graveyard widely fenced in, and when an animal is known to have anthrax, it should be placed therein and allowed to die where it is to be buried. Such an inclosure should never be used for any other purpose, and should be so located that drainage from it upon other land will not occur.

#### SUSCEPTIBILITY OF THE VARIOUS ANIMALS.

While all the farm animals and even man himself will contract anthrax, we know that the degree of susceptibility varies not only in the different species, but also in individuals of the same species. We find that the herbivora are very susceptible and that the carnivora

are less so, while the omnivora occupy a place between these two extremes. Cattle and sheep, being extremely susceptible, contract the fulminating type in nearly every case, while horses and mules frequently live several days, exhibiting the local or skin form, and some cases recover. It could probably be shown that any ruminant is more susceptible than other herbivora because of the anatomical and physiological characteristics of their respective digestive organs. In ruminants we find the immense rumen, with its moisture, heat, and alkaline reaction, an admirable incubator for bacteria. The outward passage of the contents of the rumen is slow, so there is plenty of time for anthrax spores swallowed with the food to develop into bacilli and multiply into prodigious numbers before they pass through the other two stomachs into the true or acid stomach, where some of them may be digested, but where many may pass out unharmed into the alkaline intestine and be absorbed with the food, finally entering the blood stream suspended in the chyle. It would now require only a few hours for them to multiply in the blood into sufficient numbers to produce their fatal poison. It is doubtless in this way that cattle which seem well when last seen and fed at night come down suddenly with the disease and are found dead in the morning.

Swine are less susceptible than horses and cattle, yet when swine eat the carcasses of animals dead from anthrax they may contract the disease. As in other species, the young sucking pigs are more susceptible than the adults. As swine usually contract the disease by eating the dead animals, they generally have the disease in the throat or intestine. The symptoms in such cases do not vary materially from like cases in the other species.

Dogs and cats contract the disease similarly to hogs and are classed among the less susceptible. The symptoms are those to be expected, and consist of swelling of the throat, difficult swallowing, vomiting, profuse bloody diarrhea, high fever, and death.

Birds of prey are said to be immune to the disease. Chickens are less susceptible than swine, yet Pasteur showed that chickens were quite susceptible when immersed in cold water and then inoculated. Likewise frogs, which are ordinarily immune, can be successfully inoculated after immersion in warm water. Young pigeons of certain breeds are easily infected artificially, and sparrows, finches, canaries, yellow-hammers, and redbreasts have been successfully inoculated.

In chickens anthrax runs a very rapid and fatal course in 24 hours. They can contract the disease by eating the carcass or discharge of an animal dead from anthrax. As in other animals, a differential diagnosis between this disease and other chicken diseases of like type should be based upon the discovery of the anthrax bacillus in the blood along with the symptoms of sudden debility

and high fever. Anthrax swellings will occur on the comb and wattles, around the eyes, and on the tongue, palate, and feet. The bird is extremely weak, has violent tremors and convulsions, and bloody diarrhea, and dies within 24 hours.

#### ANTHRAX IN MAN.

Anthrax is not, strictly speaking, a human disease, yet it is not rare in the practice of physicians who attend the workers in tanneries where imported hides are converted into leather, nor is it rare in persons whose occupation brings them in otherwise contact with hides, wool, hair, furs, hoofs, bones, rags, felt, glue, or any product made from the bodies of animals that die of anthrax. Hence the disease is found in shepherds, cattlemen, horsemen, farmers, drovers, butchers, tanners, brush makers, and veterinarians.

The disease is notoriously common in those who handle imported hides, although man can become infected through the bites of flies or other infected insects. The sound skin is sufficient protection against the entrance of the bacillus, but the slightest abrasion is sufficient as a point of inoculation in handling infected material, or even for inoculation by the ordinary housefly that has visited the discharges of an animal suffering with anthrax. The process of tanning does not always disinfect an anthrax hide. That hair is a medium of infection is shown by outbreaks of anthrax among brush makers. Persons who handle imported wool and rags contract the disease by inhaling the spores in the dust which arises. This spore develops into the bacillus, and a case of pulmonary anthrax is established. This form is known as woolsorters' disease, or ragpickers' disease. Those who handle bones and carcasses in fertilizer, glue, and rendering establishments located in anthrax districts are peculiarly exposed to infection. In man the large majority of cases occur on the face, probably because this part of the body is more liable to be attacked by biting insects.

Anthrax in man is a local disease, and we do not find the bacilli in as large numbers in the blood or other organs, such as the liver, spleen, and kidneys, as we do in the lower animals. The bacilli can, however, be found in abundance in the local lesion.

#### MALIGNANT PUSTULE.

Malignant pustule, or local anthrax of the skin, usually occurs on the face, hands, arms, or neck of those who handle imported hides. In from one to three days after infection takes place a small red pimple appears. This soon changes to a vesicle, which is very painful. The center of the vesicle rapidly becomes necrotic, forming a black eschar, which soon becomes surrounded by a ring of vesicles.



The surrounding tissues become congested and edematous, and the lymphatics are involved. The fever is quite high, and the patient is very sick from the absorption of poison from the local disease. It runs a rapid course, and the patient may die from toxemia.

Most of these cases are, fortunately, susceptible of successful treatment. This consists of early and thorough excision of the pustule and all infected surrounding tissue, followed by the local application of strong disinfectants. At Guy's Hospital, in London, 13 out of 15 cases were cured by excision, even though in 12 of these cases the inflammation had spread to the surrounding parts or had involved the lymphatic glands with more or less severe constitutional disturbance. In some cases the disease process goes no further than the formation of the eschar, which then becomes a scab, with subsidence of the inflammatory process, and recovery. In the majority of cases, however, the disease runs a regular course; if not excised early the bacilli enter the blood stream and the case terminates fatally from a modified form of the same disease as occurs in cattle, with the exception that the bacilli are not found in large numbers in the internal organs nor is the spleen greatly enlarged. It may, therefore, be said that in man death is largely due to the absorption of toxins from the local lesions. When the pustule is situated on the extremities the percentage of recoveries under treatment is large.

#### PULMONARY AND INTESTINAL ANTHRAX.

Pulmonary anthrax, or woolsorters' disease, is caused by the inhalation of spores by those who handle hides or other infected material. The initial lesion is local and is situated in the lower trachea. It consists of a swollen and hemorrhagic condition of the mucous membrane, with great enlargement of the mediastinal and bronchial glands and of effusions into the pleural and pericardial cavities and the lungs. Externally may occur also cutaneous edema over the chest and neck and inflamed glands. This form is rapidly fatal, the patient dying from suffocation and toxemia.

Intestinal anthrax is also of local origin and has about the same pathology and terminations as the pulmonary form. There is intense inflammation of the intestinal mucous membrane and involvement of the neighboring lymphatic glands. Intestinal infection takes place when anthrax-infected products are eaten.

#### THE ANTHRAX SEASON.

Anthrax is peculiarly a disease which may be said to be a seasonal one; that is, the disease makes its appearance with the advent of certain kinds of weather. In March, 1907, in Delaware we were visited with some exceptionally warm, springlike weather of con-



siderable duration, and on the 22d of that month the first case occurred for that year, an exceptionally early start. In 1908 the first case occurred on May 2. In 1909 the first case did not occur until July 23, but the disease persisted until November 6, whereas in 1907 the last case was on September 24, and in 1908 on August 24. So that we may say the season lasts, in Delaware, from March to November, being most prevalent in the summer months. As the disease is, generally speaking, a warm-weather one, and as we know that the causative agent is present throughout the year, there must be some conditions which come with warm weather which predispose to the onset of the disease, either by affecting the receptivity of the animals or by increasing the infective properties of the germ of the disease. Possibly both conditions prevail. There can be little doubt that the resting stage of the *Bacillus anthracis*—the spore—will quickly develop into the bacillar or infecting stage under the influence of heat, moisture, and organic matter. These conditions prevail on the pasture in warm weather, and the writer has demonstrated that the germ of anthrax will vegetate in a 2 per cent hay infusion. Hence, when spores of anthrax come in contact with hay infusions in pastures, especially in meadows, there is produced a virulent culture which will infect the animal eating them.

The animals themselves are probably more susceptible to anthrax when turned out upon fresh pastures, as the ingestion of rank grasses, which are acid in reaction, lessens the normal alkalinity of the blood and thereby increases the susceptibility to anthrax. This greater alkalinity of the blood in carnivora may be the cause of their greater resistance to the disease, while the loss of alkalinity of the blood by the herbivora, and especially by ruminants such as cattle and sheep, through the ingestion of such large quantities of acid grass, may account for their increased susceptibility during the grass-growing season. At all events there are certain weather factors which determine, in a measure, the onset of the anthrax season, and given such conditions the season can be predicted with tolerable certainty. This season exists when we have had a long, dry period, followed by light rains or infrequent heavy rains, and then by extreme heat. This kind of weather will not only produce bacterial multiplication, but will cause a rapid growth of rank acid grasses, and these when eaten will increase the susceptibility of animals to anthrax by decreasing the alkalinity of their blood.

Anthrax, then, may be said to be a pasture disease, which exists mostly in warm weather. If it were a stable disease, why should we not have cases occurring in winter, and why is it that the disease rarely occurs in animals that are not turned out on pasture? We rarely see a case of anthrax in a carriage horse that is fed in the stall, but it is not of infrequent occurrence in horses that are turned

out to pasture. Cases are rare in city horses, but common in the farm horse. The city horse must eat the same hay and grain, generally speaking, as the farm horse. If it were the hay and grain that produce the disease, we should find anthrax as common in city stalls as on the farm.

Anthrax rarely occurs in small towns. It is distinctly a disease of animals that graze on infected fields. A recent case emphasizes this. An expressman delivered some goods to a farmer who lives 1 mile from this town (Newark, Del.). This farm has anthrax every season. The expressman's horse must have grazed upon infected soil while there, as it was sick within 48 hours with high fever and edematous swellings at the root of the neck and down the front legs. A microscopic examination of the exudate showed the presence of anthrax bacilli, and the animal died in a few hours. This animal lived in the stable with others, and was fed the same feed. No case has occurred since, and it was the only animal from that stable that visited the infected farm. The great importance of an early diagnosis and isolation was demonstrated in this case. Within a few hours after a swelling was noticed anthrax was suspected and some of the bloody subcutaneous exudate was aspirated and brought to the writer by the attending veterinarian for diagnosis. It was thus possible to remove the animal from the stable to a yard where other animals could not become infected long before any infecting discharge had taken place. The animal was embalmed, washed down with formalin solution, as previously described in this article, and buried in a six-foot grave as soon as the same could be dug. The stables have been used uninterruptedly ever since (four months), and no fresh case of anthrax has occurred.

#### PREVALENCE OF ANTHRAX IN DELAWARE.

Anthrax has been officially recognized as existing in this State (Delaware) since August, 1892. It is highly probable that the disease had its origin in imported hides used in the tanneries at Wilmington for making leather. Large quantities of scraps accumulate during the process of leather making, and these were sold to farmers who composted them and later spread the infected material upon the land. Since the spores of anthrax would live and retain their virulence in a compost heap, it is easy to see how the lands first became infected. While it is generally conceded that meadow pastures are most productive of the disease, the tillable lands were probably infected first, as above stated. The meadow pastures have retained their infective properties because of their constant use for grazing, and particularly because of fresh cases of anthrax occurring upon them. The higher tillable lands have, however, in many cases become disinfected by the lapse of time, cultivation, and drainage.

Estimates made by veterinarians practicing in the State show that there are from 175 to 200 farms in Delaware that are permanently infected with anthrax. These farms are located, in a general way, in that territory which drains into the Delaware River and the Delaware Bay. The infected territory comprises about one-third of the total area of the State.

While anthrax has been known to exist in the State only since 1892, it is highly probable that the first cases occurred much earlier and that the disease is as old as the morocco-leather industry. The originally infected territory must have been the cultivated lands upon which infected compost fertilizer was spread by the farmer himself. Animals dying on such farms before the nature of the disease was known here were either hauled out dead or turned out while yet alive upon the marshes to die. Doubtless they would in some cases be set adrift in the creeks and thus infect any shore or marsh upon which they drifted. As the marshes are always wet and contain decaying vegetation in abundance, the conditions for the development of the microbe of anthrax are perfect, while upon the dry cultivated lands, even though the microbe be present, the conditions are, owing to the comparative absence of water, much more unfavorable to the multiplication of the causative agent. It is because of these conditions that marsh pastures are regarded as being more productive of the disease. The wash water from the tanneries at Wilmington can no doubt infect the streams into which it flows and cause the disease in animals either grazing upon them or drinking from them.

Those farms and marshes now infected will remain so for many years, even though no new cases occur upon them. This liability to outbreaks of anthrax on a farm has a depressing effect, not only upon the value of the land, but upon all agricultural operations.

While there has been no epidemic of anthrax in Delaware for several years, and the number of deaths from the disease has been exceeded by many ordinary diseases of which little notice is taken, anthrax must always be considered a menace to the agricultural welfare of the State because of the ever-present liability of an outbreak which might assume the proportion of a genuine epidemic. This liability should be offset by the annual vaccination of every farm animal in the infected territory. Even though several years may have elapsed since the last case occurred on a farm, that farm is liable at any time to be again visited by the disease.



## METHODS OF PRODUCING IMMUNITY IN ANIMALS.

## THE EXISTING METHOD OF DOUBLE VACCINATION, (PASTEUR METHOD).

Since 1892 anthrax has been controlled by vaccination by a method devised by Louis Pasteur. This method consists in the subcutaneous injection of attenuated cultures of the bacillus of anthrax. Two injections of varying degrees of strength are made at an interval of 12 to 14 days. The first injection consists of 1 cubic centimeter of a culture that has been incubated at  $42^{\circ}$  to  $43^{\circ}$  C. for a sufficient time to decrease its virulence to a point where it will kill white mice, but not guinea pigs or rabbits. This generally requires a period of 24 days, assuming that the culture was made originally from a moist, virulent race of anthrax bacilli. Such cultures are to be made directly from the heart's blood of an animal that has died of anthrax within 48 hours after inoculation. In such blood we find only non-spore-bearing bacilli, and when these are grown at  $42^{\circ}$  to  $43^{\circ}$  C. they do not at any time produce spores while this temperature is maintained. Contrary to the statements of some, however, the bacilli promptly form spores when this temperature is reduced. These spores do not acquire any more virulence, however, than the parent bacilli which produced them.

The second injection consists of a culture similarly made and incubated at  $42^{\circ}$  to  $43^{\circ}$  C. for a period of 12 to 18 days, or one whose virulence has been reduced to a point where it will not kill rabbits but will kill white mice and guinea pigs, the latter in 3 or 4 days. The author's experience has shown that there is no hard and fast line in the number of days that a culture of a given race of anthrax bacilli must be attenuated. Much depends upon the resistance of the bacillus, the character of the culture medium, the exactness of the temperature of the incubator, and the natural resistance of the animals used in testing the vaccines. When a culture of proper strength has been obtained and is properly transferred to fresh media about once a month and kept in a cool place where it will not evaporate, it may be used indefinitely as a stock culture for inoculating a liquid medium, which constitutes the vaccine. When such attenuated cultures are inoculated into animals, a very mild and clinically unnoticeable attack of anthrax is produced, which confers an active immunity which persists throughout the anthrax season. The inoculation must be repeated the following season.

Certain precautions are necessary in the application of anthrax vaccine. Assuming that the vaccine has been properly prepared, it is the duty of the veterinarian to ascertain that anthrax is not already existing in the animals he is about to vaccinate. As a precaution, when there is reason to suspect that anthrax may already



be existing, no animal showing a fever should be vaccinated, as the disease may be carried from the already infected animal to others upon the point of the inoculating needle. Again, in order to prevent abscess in horses and mules by introducing under the skin streptococci or staphylococci by the point of the needle, the place of injection should be disinfected of these microbes. The anthrax bacillus can not produce pus, but the germs usually found upon the skin are to be held responsible when abscess occurs from vaccination. The writer has been in the habit of dipping the point of the needle into strong carbolic acid contained in a small vial which may be conveniently carried in the side pocket at the time of vaccinating. None of this acid can enter the needle and kill the vaccine germs, as it is already filled with the vaccine, and none can be introduced under the skin, as it is all removed from the needle in its passage through the skin, and this in turn disinfects the wound made. While every veterinarian knows that hypodermic injections of medicine are daily made with a minimum of abscess production, it must be recognized that the conditions are not similar, and hence it is deemed highly advisable that these precautions be taken, especially in horses and mules.

There is usually little or no swelling at the point operated upon. Where abscess occurs the operator is to blame. It means that pus-producing germs have been carried in upon the needle and that either the needle or the skin was not disinfected. Abscess is more likely to occur in horses and mules than in other animals.

It has been the practice in Delaware to continue the vaccinated animals at their usual work. We have no data upon the subject showing this is unwise, but it is believed that the animals should be shielded as much as possible from excessive work and from extremes of heat or cold or from chilling rains. We advise our vaccinators, who consist of regular, practicing veterinarians designated by the governor upon the recommendation of the board of agriculture, to destroy all opened bottles of vaccine that remain unused at the end of a day's work, as it will certainly become contaminated and be spoiled if kept overnight at ordinary temperatures. The vaccine is dispensed in 50-dose bottles hermetically sealed and distinctively labeled, so there can be no mistake made in using it.

#### EFFECTIVENESS OF THE METHOD.

The results of vaccination have been as good, as shown by our statistics, as those obtained by the use of any other biological product. That the method is entirely safe is shown by the fact that only one dangerous swelling has been brought to our notice in three years' experience in Delaware. Every year there are instances where, for

some reason, animals that have been vaccinated die from anthrax, showing that they were not protected by the vaccine. No doubt some of these failures may be due to the animal being missed during vaccinating. In other cases the failure may be due to the vaccine needle not properly puncturing the skin, so that the vaccine falls upon the ground. These accidents are readily brought about when the animals are unruly. On the other hand, the writer has observed a case of anthrax that occurred in vaccinated animals—that is, where it was positively certain that the vaccine was properly prepared and active and had been properly applied.

Some cases of nonprotection by vaccination can be explained by the fact that the vaccine itself was inert, and therefore nonprotective. Cultures made from such vaccine failed to grow. The vaccine would, of course, fail in the usual physiological test for an anthrax vaccine, and, of course, would fail to protect the animal against anthrax. The writer has known of instances where for various reasons an animal would escape vaccination, and that animal would be the only one to die on that farm. Hence he feels warranted in highly commending vaccination as the most important means of combating this terrible scourge. In France, where vaccination is most popular, and where statistics are reliable because of governmental control, the death rate before vaccination was adopted was, in cattle 5 per cent, and in sheep 10 per cent. After vaccination was adopted the losses were reduced to 0.34 per cent in cattle and 0.94 per cent in sheep. In Delaware the losses in 1907 in horses and cattle that were vaccinated were 0.32 per cent.

Vaccination should be practiced every spring, at least a month before it is time to turn the animals out to pasture, as a month is required for the production of immunity. This vaccinating should not be optional with the owner, as at present, but should be compulsory, the State assuming the risk of loss from the use of the vaccine, but not in those cases where it can be shown the animal died not from vaccination but from a natural infection, owing to failure of being protected by the vaccine, or from other causes.

#### A TEST OF PASTEUR VACCINES.

Along with the investigation of anthrax for the purpose of discovering new methods of treatment and prevention of the disease, and for studying the biology of the causative organism and the general sanitary aspects of the subject, it was decided to test the efficacy of freshly prepared Pasteur vaccines. It is believed that some of the bad results that have been reported as following the use of Pasteur vaccine is due to carelessness on the part of those preparing and using it. As there is no visible difference in the appearance of the two

vaccines, Nos. 1 and 2, it is an easy matter to get the bottles mixed or improperly labeled. If the labels should come off the bottles, the vaccinator would have no guide as to which vaccine he was using. The writer has purchased anthrax vaccines on the open market and has found some that were wholly inert, and others that were too strong when tested in the usual way.

For the purpose of gaining such information as is possible from the practical experiences of practicing veterinarians who vaccinate against anthrax in Delaware, the writer undertook to prepare Pasteur vaccines for the State board of agriculture, which were to be used as soon as possible after they had reached destination. The cultures constituting the vaccines were grown in 50-dose bottles, each bottle containing 50 c. c. of bouillon which had been inoculated 24 hours previously with the vaccinal germs. The official vaccinator was furnished first with No. 1 vaccine. In 12 days he was shipped the No. 2 vaccine, for use on the same animals. Under this plan it was impossible for the bottles of vaccine to get mixed, even though the labels came off.

The plan of using fresh vaccine in which the germ is still in the bacillar stage was continued with good results for two seasons. For the last two years the plan of using vaccines that had been incubated for four or five days, or until the bacilli had spored, has been tried. This latter method represents the condition of vaccine as put on the market by commercial houses, and is a suspension of spores instead of bacilli. According to the experiences of these two seasons, we have reason to believe that the vaccine will remain active for several months, and such cultures may be prepared several months in advance of their use, provided the incubation is carried to a point where all growth ceases, or spores form. These spores inherit just that degree of strength possessed by their progenitors, and do not change except with a considerable lapse of time, possibly a year, if kept under favorable conditions. It is highly important that anaerobic conditions be not established in the bottles, as the bacilli will sink to the bottom and will not spore when grown anaerobically. To prevent this, it is important that the culture medium be made to reabsorb the air which has been driven out of it during sterilization before being inoculated with the vaccine germs. In preserving stock cultures of the vaccine it is very important that they be incubated for several days before being taken out of the incubator. If they are removed before spores have formed, the more vulnerable bacilli may succumb to existing unfavorable conditions. To these conditions may be ascribed the death of cultures of anthrax vaccine, and the loss of virulence of former virulent cultures of anthrax bacilli, which sometimes occur during the course of laboratory work.



**EXPERIMENTS WITH VARIOUS SUBSTANCES TO TEST IMMUNIZING POWERS.**

Experiments upon the disease have been carried on uninterruptedly for the past three years. These had for their object the preparation of substances in the laboratory and in living animals which could be used in combating anthrax by acting as antitoxins or bactericides, or as vaccines.

Realizing the great importance and economy in producing protective and curative substances in the laboratory over the necessarily expensive methods when employing animals for the same purposes, efforts have been made along the line of producing various culture products to be used in combating anthrax.

Pyocyanase, made after the method of Emmerich and Loew, gave some good results when tested upon rabbits, but failed upon sheep.

Anthraxase, prepared by the writer after the same general method used in producing pyocyanase, was without protective or curative properties, although it produced high fever when injected into rabbits and sheep subcutaneously.

Anthraxoin, consisting of a turbid suspension of dead anthrax bacilli, was apparently useless in protecting sheep against anthrax when used similarly to the Pasteur vaccine.

A single vaccine, having for its object the production of immunity in two weeks and thus cutting down the necessary period by one-half, was made by incubating a virulent bacillus for about 18 days at 42° to 43° C. Such a culture will kill guinea pigs in about a week, and in strength it thus holds a position between the two vaccines of Pasteur. With it sheep were vaccinated and after 12 days withstood an otherwise fatal infection with virulent bacilli. In some cases, however, the immunity was not sufficiently strong, as was evidenced by the death, now and then, of a sheep when tested with virulent bacilli.

The preparation and use of the various substances, together with the results of the experimental work, are described in the following pages.

**ANTHRAXIN.**

Anthraxin was made similarly to tuberculin and mallein. The cultures of anthrax were grown for 10 days with daily shaking in glycerinated, peptonized bouillon, the glycerin being used in 4 per cent strength. The cultures were then sterilized by boiling, filtered through Berkefeld filters, and then evaporated to one-tenth of the original volume. A sirupy liquid, much resembling tuberculin, resulted. It was tested on rabbits and sheep for immunizing properties, as shown in Table 1, on the following page.



TABLE 1.—*Experiments with anthraxin.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxin.	Virulent culture anthrax.		
Rabbit 1.....	1907.			° F.	
	July 10.....	1 c. c. subcutaneous.			
	July 17, 10 a. m...			105, 3 p. m...	
	July 18.....			104, 10 a. m...	
	.....do.....			103, 3 p. m...	
	July 19.....			Normal.....	
	July 24, 11 a. m...	1 c. c. subcutaneous.		104.....	
	July 24, 1.30 p. m...			105.....	
	July 24, 5.30 p. m...			105.....	
	July 25, 9 a. m...			Normal.....	
	July 31, 9 a. m...		0.05 c. c.		
	Aug. 2, 9 a. m...				Dead from anthrax.
Sheep 1.....	Aug. 27, 1 p. m...	5 c. c. subcutaneous.			
	Aug. 27, 6 p. m...				
	Aug. 28, 9 a. m...				Sheep blowing, head down.
	Sept. 3, 9 a. m...	5 c. c. subcutaneous.			Sheep seems well.
	Sept. 10, 9 a. m...		0.25 c. c.		
	Sept. 12, 9 a. m...				Dead from anthrax.
Sheep 2.....	Aug. 27, 1 p. m...	5 c. c. subcutaneous.			
	Aug. 27, 6 p. m...				
	Aug. 28, 9 a. m...				Sheep blowing, head down.
	Sept. 3, 9 a. m...	5 c. c. subcutaneous.			Seems well.
	Sept. 10, 9 a. m...	.....do.....			
	Sept. 23, 9 a. m...		0.25 c. c.		
	Sept. 25, 9 a. m...				Dead from anthrax.
Sheep 3.....	Aug. 27, 9 a. m...	5 c. c. subcutaneous.			
	Aug. 27, 6 p. m...				Blowing; no fever.
	Sept. 3, 9 a. m...	5 c. c. subcutaneous.			
	Sept. 5.....				Diarrhea.
	Sept. 10, 9 a. m...	5 c. c. subcutaneous.			Well.
	Sept. 15, 9 a. m...		0.25 c. c.		
	Sept. 17, 9 a. m...				Dead from anthrax.
Sheep 4.....	Aug. 27, 9 a. m...	5 c. c. subcutaneous.			
	Aug. 11, 6 p. m...				Blowing; no fever.
	Sept. 13, 9 a. m...	5 c. c. subcutaneous.			Diarrhea for several days.
	Sept. 10, 9 a. m...	.....do.....			
	Sept. 15, 9 a. m...		0.25 c. c.		
Sheep 5 and 6...	Sept. 17, 9 a. m...				Dead from anthrax.
	Sept. 3, 9 a. m...	5 c. c. subcutaneous.			
	Sept. 6.....				Seemed well.
	Sept. 10, 9 a. m...	5 c. c. subcutaneous.			
	Oct. 7, 9 a. m...		0.25 c. c.		
	Oct. 9, 9 a. m...				Both dead from anthrax.

From the foregoing experiments we gather that anthraxin was possessed of no immunizing properties whatever.

## PYOCYANASE.

Pyocyanase, the next substance experimented with, was prepared as follows: Large flasks of medium were inoculated with *Bacillus pyocyaneus* and grown at 37° C. until a ropy condition was produced, which required three weeks, the flasks meanwhile being shaken daily. The composition of the medium was as follows: Peptone, 0.5 per cent; glycerin, 0.1 per cent; dipotassium phosphate, 0.1 per cent; magnesium sulphate, 0.01 per cent; sodium chlorid, 0.3 per cent; sodium bicarbonate, 0.1 per cent; in distilled water (synthetic medium of Emmerich and Loew). When growth had ceased in this medium the growths from a number of agar-agar cultures of the same organism were added and the whole was thoroughly shaken. The culture medium was then neutralized with dilute hydrochloric acid. Carbolie acid was then added to 0.2 per cent strength as a preservative. The liquid was then evaporated down to one-tenth of its original volume at ordinary room temperature by being placed in pie plates. Then the liquid was dialyzed for 24 hours in running water, filtered through Berkefeld filters, and 0.2 per cent carbolie acid was again added to replace that which was dialyzed out. The resulting liquid, pyocyanase, has a dark coffee color and a pungent odor. The experiments to test its immunizing and curative properties upon guinea pigs, rabbits, and sheep are recorded in Tables 2 and 3.

TABLE 2.—Experiments with unfiltered pyocyanase.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture anthrax.		
Rabbit 1.....	1907. Dec. 9, 9 a. m....	6 c. c. sub-cutaneous.		° F.	Rabbit dead. Liver necrosed, spleen enlarged; anthrax bacilli at local lesion, but none found in blood or in liver and spleen; probably died from pyocyanase poisoning.
	Dec. 10, 9 a. m....		0.2 c. c. sub-cutaneous.		
	Dec. 11, 9 a. m....	4 c. c. sub-cutaneous.			
Guinea pig 1.....		3 c. c. sub-cutaneous.			Died during night. No bacilli in blood, but few at local lesion; probably died from pyocyanase poisoning.
	Dec. 17, 10 a. m....		0.1 c. c. sub-cutaneous.		
	Dec. 18, 10 a. m....	1½ c. c. sub-cutaneous.			
	Dec. 18, 1.30 p. m....	2 c. c. sub-cutaneous.			
Guinea pig 2....	Dec. 16, 10 a. m....	3 c. c. sub-cutaneous.			
	Dec. 17, 10 a. m....		0.1 c. c. sub-cutaneous.		

TABLE 2.—*Experiments with unfiltered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture anthrax.		
Guinea pig 2...	1907. Dec. 18, 9 a. m....	1½ c. c. sub-cutaneous.	.....	° F.	Sick.
	Dec. 19, 9 a. m....	.....	.....	.....	Chloroformed. No bacilli in blood, but a few at local lesion; pyocyanase poisoning.
Guinea pig 3...	Dec. 19, 9 a. m....	3 c. c. sub-cutaneous.	.....	.....	
	Dec. 20, 10 a. m....	do.	.....	.....	
	Dec. 21, 3 p. m....	1½ c. c. sub-cutaneous.	.....	.....	
	1908. Jan. 2, 9 a. m....	.....	0.1 c. c. sub-cutaneous.	.....	
Rabbit 2.....	Jan. 4, 9 a. m....	.....	.....	.....	Dead from anthrax. Bacilli in blood; another guinea pig treated similarly died of anthrax on third day after inoculation.
	Jan. 13 9 a. m....	.....	0.2 c. c. sub-cutaneous.	.....	
	Jan. 14, 9 a. m....	6 c. c. sub-cutaneous.	.....	103	
	Jan. 15, 9 a. m....	do.	.....	102	Found dead of anthrax. Bacilli in blood.
Rabbit 3.....	Jan. 16, 9 a. m....	.....	.....	.....	
	Jan. 13, 9 a. m....	6 c. c. sub-cutaneous.	.....	.....	
	Jan. 14, 9 a. m....	.....	0.3 c. c. sub-cutaneous.	.....	Edema at points of injection of pyocyanase, no fever.
	Jan. 15, 9 a. m....	6 c. c. sub-cutaneous.	.....	Normal.	
Rabbit 4.....	Jan. 18, 9 a. m....	.....	.....	.....	Animal apparently well.
	Jan. 25, 9 a. m....	.....	.....	.....	Animal dies from anthrax on eleventh day. Bacilli in blood.
	Jan. 13 9 a. m....	6 c. c. sub-cutaneous.	0.2 c. c. sub-cutaneous.	.....	
	Jan. 14, 9 a. m....	do.	.....	Normal.	
Rabbit 5.....	Jan. 15, 9 a. m....	do.	.....	do.	
	Jan. 16, 9 a. m....	.....	.....	.....	Dead from anthrax. Another rabbit treated similarly died of anthrax on third day also; bacilli in blood.
	Feb. 3, 10 a. m....	½ c. c. sub-cutaneous.	.....	.....	
	Feb. 4, 10 a. m....	do.	0.3 c. c. sub-cutaneous.	102.8	Slight swelling at point where pyocyanase was injected.
Rabbit 6.....	Feb. 5, 10 a. m....	do.	.....	.....	
	Feb. 8, 10 a. m....	.....	.....	.....	Animal dead from anthrax, having lived four days.
	Feb. 3, 10 a. m....	1 c. c. sub-cutaneous.	.....	.....	
	Feb. 4, 10 a. m....	do.	0.3 c. c. sub-cutaneous.	.....	Considerable reaction at point of injections.
Rabbit 7.....	Feb. 5 10 a. m....	do.	.....	.....	
	Feb. 7, 10 a. m....	.....	.....	.....	Rabbit dead from anthrax on third day.
	Feb. 3, 10 a. m....	2 c. c. sub-cutaneous.	.....	.....	
	Feb. 4, 10 a. m....	do.	0.3 c. c. sub-cutaneous.	103	Small reaction from injections.
	Feb. 5, 10 a. m....	.....	.....	103	
	Feb. 8, 10 a. m....	.....	.....	.....	Animal dead from anthrax, having lived four days.

TABLE 2.—*Experiments with unfiltered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture anthrax.		
Rabbit 8.....	1908. Feb. 3, 10 a. m....	4 c. c. sub-cutaneous.	.....	° F.	Some reaction from injections. Abscess formed at point of injection. Animal dead from anthrax.
	Feb. 4, 10 a. m....	1 c. c. sub-cutaneous.	0.3 c. c. sub-cutaneous.	103.5	
	Feb. 7, 10 a. m....	.....	.....	.....	
Rabbit 9.....	Feb. 3, 10 a. m....	5 c. c. sub-cutaneous.	.....	.....	Severe reaction at point of injection. Abscess forms. Animal dead from anthrax on fourth day.
	Feb. 4, 10 a. m....	2 c. c. sub-cutaneous.	0.2 c. c. sub-cutaneous.	.....	
	Feb. 5, 10 a. m....	.....	.....	104	
	Feb. 8, 10 a. m....	.....	.....	105	

The foregoing experiments were made with unfiltered pyocyanase. The dead bacilli produce abscesses at points of injection and frequently disease of the liver, which contributed to death from anthrax.

TABLE 3.—*Experiments with filtered pyocyanase.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture of anthrax.		
Sheep 1.....	1909. Jan. 4, 10 a. m....	10 c. c. sub-cutaneous.	.....	° F. 103.6	Animal sick. Off feed. Dead from pyocyanase poisoning.
	Jan. 4, 1 p. m....	.....	.....	105	
	Jan. 4, 3 p. m....	10 c. c. sub-cutaneous.	0.1 c. c. sub-cutaneous.	106	
	Jan. 5, 9 a. m....	.....	.....	103.6	
	Jan. 5, 4 p. m....	.....	.....	103	
	Jan. 5, 7 p. m....	.....	.....	.....	
Sheep 2.....	Jan. 6, 9 a. m....	3 c. c. sub-cutaneous.	.....	103	Respirations jerky.
	Jan. 6, 12 m....	.....	.....	105.5	
	Jan. 6, 4 p. m....	.....	.....	106.9	
	Jan. 7, 10 a. m....	1 c. c. sub-cutaneous.	.....	104	
	Jan. 7, 11 a. m....	.....	.....	.....	
	Jan. 21, 3 p. m....	5 c. c. sub-cutaneous.	.....	.....	
	Jan. 22, 9 a. m....	4 c. c. sub-cutaneous.	.....	103.5	
	Jan. 22, 3 p. m....	2 c. c. sub-cutaneous.	.....	102.5	
	Jan. 23, 9 a. m....	3 c. c. sub-cutaneous.	.....	102.5	
	Jan. 23, 3 p. m....	2 c. c. sub-cutaneous.	0.1 c. c. sub-cutaneous.	103	
	Jan. 25, 9 a. m....	.....	.....	102	
	Jan. 26, 12 m....	.....	.....	102	
Sheep 3.....	Jan. 28, 10 a. m....	.....	.....	.....	Dead from anthrax, having lived 5 days.
	Jan. 7, 10 a. m....	2 c. c. sub-cutaneous.	.....	102	
	Jan. 7, 4 p. m....	.....	.....	107.2	
	Jan. 13, 9 a. m....	2 c. c. sub-cutaneous.	.....	101	
	Jan. 13, 3 p. m....	5 c. c. sub-cutaneous.	0.1 c. c. sub-cutaneous.	101	Animal does not seem sick even though temperature is high.



TABLE 3.—*Experiments with filtered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture of anthrax.		
Sheep 3.....	1909. Jan. 14, 9 a. m.....	2 c. c. subcutaneous.	.....	°F. 102.5	Anthrax fever setting in.  Dead from anthrax (3 days).
	Jan. 14 1.30 p. m.....	.....	.....	102.5	
	Jan. 15, 10 a. m.....	2 c. c. subcutaneous.	.....	104.5	
	Jan. 16.....	.....	.....	.....	
Sheep 4.....	Jan. 8, 10 a. m.....	1 c. c. subcutaneous.	.....	101	Dead two hours later of anthrax (3 days).
	Jan. 8, 1 p. m.....	.....	.....	102	
	Jan. 8, 3 p. m.....	1 c. c. subcutaneous.	0.1 c. c. subcutaneous.	.....	
	Jan. 9, 10 a. m.....	do	.....	103	
	Jan. 10, 4.30 p. m.....	do	.....	103.5	
	Jan. 11, 9 a. m.....	.....	.....	105.5	
Rabbit 10.....	1908 Mar. 5, 9 a. m.....	0.05 c. c. subcutaneous.	.....	103.3	Rabbit well.
	Mar. 6, 9 a. m.....	0.5 c. c. subcutaneous.	.....	103	
	Mar. 7, 9 a. m.....	do	.....	.....	
	Mar. 8, 9 a. m.....	.....	0.2 c. c. subcutaneous.	.....	
	Mar. 9, 9 a. m.....	0.5 c. c. subcutaneous.	.....	102.8	
	Mar. 13, 9 a. m.....	.....	.....	.....	
Rabbit 11.....	Mar. 18, 9 a. m.....	.....	.....	.....	Found dead of anthrax (10 days).
	Mar. 5, 9 a. m.....	1 c. c. subcutaneous.	.....	.....	
	Mar. 6, 9 a. m.....	do	.....	103	Animal sick.  Animal much better. Much emaciated and swollen along belly. Dead from anthrax. Post-mortem examination showed linear abscess in liver; repeated larger doses of pyocyanase probably caused animal to succumb to anthrax (lived 5 weeks); check rabbit died in 48 hours after inoculation.
	Mar. 7, 9 a. m.....	do	0.2 c. c. subcutaneous.	.....	
	Mar. 8, 9 a. m.....	.....	.....	103	
	Mar. 10, 9 a. m.....	.....	.....	101.5	
	Mar. 18, 9 a. m.....	1.5 c. c. subcutaneous.	.....	102.6	
	Mar. 21, 9 a. m.....	3 c. c. subcutaneous.	.....	103.7	
	Mar. 23, 9 a. m.....	5 c. c. subcutaneous.	.....	103	
	Mar. 24, 9 a. m.....	.....	.....	103	
	Apr. 12, 9 a. m.....	.....	.....	.....	
	Apr. 13, 9 a. m.....	.....	.....	.....	
Rabbit 12.....	Mar. 5, 9 a. m.....	1.5 c. c. subcutaneous.	.....	102	Animal has kept well.
	Mar. 6, 9 a. m.....	do	.....	102	
	Mar. 7, 9 a. m.....	do	0.2 c. c. subcutaneous.	103	
	Mar. 8, 9 a. m.....	do	.....	102.8	
	Mar. 18, 9 a. m.....	do	.....	102.8	
	Mar. 20, 9 a. m.....	3 c. c. subcutaneous.	.....	103	
	Mar. 24, 9 a. m.....	5 c. c. subcutaneous.	.....	104.3	
	Apr. 12, 9 a. m.....	.....	0.1 c. c. subcutaneous.	.....	
Rabbit 13.....	Apr. 14, 9 a. m.....	.....	.....	.....	Animal found dead from anthrax, showing immunity was only passive (lived 24 days).
	Mar. 18, 9 a. m.....	5 c. c. subcutaneous.	.....	.....	
	Mar. 18, 2 p. m.....	do	0.2 c. c. subcutaneous.	.....	

TABLE 3.—*Experiments with filtered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture of anthrax.		
Rabbit 13.....	1908.			°F.	
	Mar. 18, 4 p. m.....	.....	.....	104	
	Mar. 19, 9 a. m.....	.....	.....	104	
	Mar. 20, 9 a. m.....	5 c. c. sub-cutaneous.	.....	101	
	Mar. 28.....	.....	.....		Rabbit found dead from anthrax. Period of incubation lengthened to 10 days by pyocyanase; two more rabbits similarly treated reacted the same, and lived 10 days and 7 days, respectively.
Rabbit 14.....	Nov. 24, 9 a. m.....	1 c. c. sub-cutaneous.	.....		Simultaneous injections of "ase" and bacilli.
	Nov. 24, 3 p. m.....	do.....	0.2 c. c. sub-cutaneous.	103	
	Dec. 4, 9 a. m.....	do.....	.....		
	Dec. 5.....	.....	.....		
	Dec. 16.....	3 c. c. sub-cutaneous.	.....		
	Feb. 4.....	.....	.....		Found dead from anthrax. Lived 21 days.
Rabbit 15.....	Nov. 24, 11 a. m.....	2 c. c. sub-cutaneous.	.....		
	Nov. 24, 3 p. m.....	do.....	0.2 c. c. sub-cutaneous.	104	
	Dec. 4, 9 a. m.....	do.....	.....		Some swelling which disappears in 2 days. Animal has remained well up to Feb. 24, when it was killed by dog (lived 69 days).
	Dec. 5.....	.....	.....		
	Dec. 16.....	3 c. c. sub-cutaneous.	.....		
	Feb. 4.....	.....	.....		
Rabbit 16.....	Nov. 24, 11 a. m.....	3 c. c. sub-cutaneous.	.....		
	Nov. 24, 3 p. m.....	do.....	0.2 c. c. sub-cutaneous.	104	Animal remains well.
	Dec. 4, 9 a. m.....	do.....	.....		
	Dec. 5, 9 a. m.....	.....	.....	104.8	Swollen from "ase" injection.
	Dec. 16, 9 a. m.....	3 c. c. sub-cutaneous.	.....		
	Feb. 4.....	.....	.....		Animal has remained well up to Feb. 4, when it was killed by dog (lived 69 days). No lesions on post-mortem; check rabbit died in 48 hours after inoculation.
Rabbit 17.....	Nov. 24, 11 a. m.....	4 c. c. sub-cutaneous.	.....		Simultaneous injection.
	Nov. 24, 3 p. m.....	do.....	0.2 c. c. sub-cutaneous.	104	Chloroformed to locate position and stage of development of bacilli. No spores or bacilli found by microscope or culture in local lesion, liver, spleen, or peritoneal cavity. Check rabbit died in 48 hours after inoculation.

From the foregoing experiments with pyocyanase it will be seen that when given in proper dose and simultaneously with virulent anthrax bacilli, the period of inoculation is greatly lengthened in rabbits. Sheep, however, seem to be very susceptible to poisoning by pyocyanase, and no immunity is conferred upon them by its use.

## ANTI-THRAXASE.

Anthraxase was made in about the same manner as pyocyanase, except that the *Bacillus anthracis* was substituted for *Bacillus pyocyaneus*. The tests of this substance include cultures grown in ordinary bouillon, cultures grown in Emmerich and Loew's medium, and experiments with precipitated anthraxase. The details are given in Tables 4, 5, and 6.

TABLE 4.—*Experiments with anthraxase—Cultures grown in ordinary bouillon.*

Animal.	Date.	Injections.		Temperature.	Results and remarks.
		Anthraxase.	Anthrax culture.		
Rabbit 18.....	1908. Mar. 17, 9 a. m....	0.75 c. c. subcutaneous.	.....	° F. 101	
	Mar. 18, 9 a. m....	0.50 c. c. subcutaneous.	.....	101.5	
	Mar. 19, 9 a. m....	.....do.....	0.2 c. c. subcutaneous.	102	
	Mar. 20, 9 a. m....	3 c. c. subcutaneous.	.....	102.4	
	Mar. 21, 9 a. m....	5 c. c. subcutaneous.	.....	107	Anthrax fever.
	Mar. 23, 9 a. m....	.....do.....	.....	.....	Animal very sick.
	Mar. 25, 9 a. m....	.....do.....	.....	.....	Dead from anthrax (lived 5 days).
Rabbit 19.....	Mar. 17, 9 a. m....	1.5 c. c. subcutaneous.	.....	100.5	
	Mar. 18, 9 a. m....	.....do.....	.....	102	Swollen lymphatics.
	Mar. 19, 9 a. m....	2 c. c. subcutaneous.	0.2 c. c. subcutaneous.	102.6	
	Mar. 20, 9 a. m....	3 c. c. subcutaneous.	.....	104.6	Anthrax fever.
	Mar. 21, 9 a. m....	.....do.....	.....	.....	Dead from anthrax on second day.
Rabbit 20.....	Mar. 17, 9 a. m....	1 c. c. subcutaneous.	.....	101	
	Mar. 18, 9 a. m....	.....do.....	.....	101.5	
	Mar. 19, 9 a. m....	.....do.....	0.2 c. c. subcutaneous.	.....	
	Mar. 20, 9 a. m....	.....do.....	.....	104.1	Anthrax fever.
	Mar. 21, 9 a. m....	3 c. c. subcutaneous.	.....	103	
Rabbit 21.....	Mar. 22, 9 a. m....	.....do.....	.....	.....	Dead from anthrax on third day.
	Mar. 23, 9 a. m....	0.5 c. c. subcutaneous.	.....	103	Animal pregnant.
	Mar. 24, 9 a. m....	.....do.....	.....	104	
	Mar. 26, 9 a. m....	5 c. c. intravenously.	.....	104	
	.....do.....	3 c. c. subcutaneous.	0.2 c. c. subcutaneous.	.....	Animal died from shock.
Rabbit 22.....	Mar. 23, 9 a. m....	2 c. c. subcutaneous.	.....	104.5	
	Mar. 24, 9 a. m....	.....do.....	.....	104	
	Mar. 26, 9 a. m....	5 c. c. intravenously.	0.2 c. c. subcutaneous.	103.1	
	.....do.....	3 c. c. subcutaneous.	.....	.....	
	Mar. 27, 9 a. m....	.....do.....	.....	102.9	
	Mar. 28, 9 a. m....	4 c. c. subcutaneous.	.....	102.4	
	Mar. 30, 9 a. m....	.....do.....	.....	.....	Died of anthrax on fourth day.
Rabbit 23.....	Mar. 23, 9 a. m....	4 c. c. subcutaneous.	.....	105	Animal excited.
	Mar. 24, 9 a. m....	.....do.....	.....	106.5	
	Mar. 25, 9 a. m....	.....do.....	.....	.....	
	Mar. 26, 9 a. m....	5 c. c. subcutaneous.	0.2 c. c. subcutaneous.	103.4	



TABLE 4.—*Experiments with anthraxase, etc.*—Continued.

Animal.	Date.	Injections.		Temperature.	Results and remarks.
		Anthraxase.	Anthrax culture.		
Rabbit 23 .....	1908. Mar. 28, 9 a. m. ....	4 c. c. subcutaneous.	.....	° F.	Died of anthrax on third day. Animal pregnant.
	Mar. 30, 9 a. m. ....	.....	.....	.....	
Rabbit 24 .....	Mar. 23, 9 a. m. ....	6 c. c. subcutaneous.	.....	104.1	
	Mar. 24, 9 a. m. ....	.....do.....	.....	105	
	Mar. 25, 9 a. m. ....	.....do.....	.....	103.9	
	Mar. 26, 9 a. m. ....	5 c. c. intravenously.	.....	.....	
	.....do.....	3 c. c. subcutaneous.	0.2 c. c. subcutaneous.	.....	
Rabbit 25 .....	Mar. 28, 9 a. m. ....	4 c. c. subcutaneous.	.....	103.6	Dead from anthrax on fourth day. Animal pregnant.
	Mar. 30, 9 a. m. ....	.....	.....	.....	
	Mar. 25, 9 a. m. ....	3 c. c. intravenously.	0.1 c. c. subcutaneous.	103	
	Mar. 26, 9 a. m. ....	5 c. c. intravenously.	.....	103.8	
	.....do.....	3 c. c. subcutaneous.	.....	.....	
Rabbit 26 .....	Apr. 7, 9 a. m. ....	0.5 c. c. subcutaneous.	.....	.....	
	Apr. 8, 9 a. m. ....	.....do.....	.....	102	
	Apr. 9, 9 a. m. ....	.....do.....	0.1 c. c. subcutaneous.	103	
	Apr. 10, 9 a. m. ....	.....do.....	.....	.....	
	Apr. 11, 9 a. m. ....	2 c. c. subcutaneous.	.....	105	
	Apr. 11, 4 p. m. ....	3 c. c. subcutaneous.	.....	105.3	
	Apr. 12, 9 a. m. ....	.....	.....	.....	

TABLE 5.—*Experiments with anthraxase. Cultures grown in Emmerich and Locie's medium.*

Animal.	Date.	Injections.		Temperature.	Results and remarks.
		Anthraxase.	Anthrax culture.		
Rabbit 27 .....	1908. Apr. 7, 10 a. m. ....	0.5 c. c. subcutaneous.	.....	° F. 102	Much swollen. Dead from anthrax on fifth day.
	Apr. 9, 10 a. m. ....	.....do.....	0.2 c. c. subcutaneous.	102	
	Apr. 10, 10 a. m. ....	.....do.....	.....	102	
	Apr. 11, 10 a. m. ....	1 c. c. subcutaneous.	.....	102.3	
	Apr. 11, 4 p. m. ....	3 c. c. subcutaneous.	.....	102.5	
	Apr. 12, 4 p. m. ....	.....	.....	103	
	Apr. 13, 4 p. m. ....	.....	.....	.....	
Rabbit 28 .....	Apr. 15, 10 a. m. ....	0.25 c. c. subcutaneous.	.....	.....	Dies of anthrax in 2 days.
	Apr. 16, 10 a. m. ....	.....do.....	.....	100.8	
	Apr. 17, 10 a. m. ....	.....do.....	.....	99.5	
	Apr. 18, 10 a. m. ....	.....do.....	.....	99.8	
	Apr. 25, 10 a. m. ....	.....do.....	0.2 c. c. subcutaneous.	101	
	Apr. 26, 10 a. m. ....	0.50 c. c. subcutaneous.	.....	102.8	
	Apr. 27, 10 a. m. ....	.....	.....	.....	

The following experiments on rabbits and sheep were made with precipitated anthraxase. Absolute alcohol, 9 volumes to 1 of anthraxase solution, was employed as the precipitant. The resulting yellow, gummy precipitate was readily soluble in water, to which 0.2 per cent strength of carbolic acid was added.

TABLE 6.—*Experiments with precipitated anthraxase.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxase.	Anthrax culture.		
Rabbit 29.....	1908.			° F.	
	Apr. 15, 9 a. m. ....	0.5 c. c. sub-cutaneous.		101	
	Apr. 16, 9 a. m. ....	do.		101	
	Apr. 17, 9 a. m. ....	do.		101	
	Apr. 18, 9 a. m. ....	do.		101	
	Apr. 25, 9 a. m. ....	do.	0.01 c. c. sub-cutaneous.		
	Apr. 26, 9 a. m. ....	do.		101.8	
	Apr. 27, 9 a. m. ....	do.		102	Check rabbit dies.
	Apr. 27, 5 p. m. ....	5 c. c. sub-cutaneous.			
	Apr. 28, 9 a. m. ....			103	
	Apr. 29, 9 a. m. ....			103	
	Apr. 30.....				Animal seems perfectly well.
	Nov. 5, 9 a. m. ....		0.1 c. c. sub-cutaneous.		Given to test duration of immunity.
	Nov. 9, 9 a. m. ....				Found dead of anthrax.
Rabbit 30.....	May 2, 10 a. m. ....	1 c. c. sub-cutaneous.		103.3	
	May 2, 2 p. m. ....	do.		102.5	
	May 2, 4 p. m. ....	do.	0.1 c. c. sub-cutaneous.	103	
	May 4, 10 a. m. ....	3 c. c. sub-cutaneous.		102	
	May 4, 4 p. m. ....			104.5	
	May 5, 11.30 a. m. ....	2 c. c. sub-cutaneous.		105	
	May 5, 4 p. m. ....			104	
	May 6, 10 a. m. ....			100.3	Dead of anthrax at 4 p. m. on fifth day.
Rabbit 31.....	May 2, 10 a. m. ....	1 c. c. sub-cutaneous.		102.3	
	May 2, 2 p. m. ....	do.		101	
	May 2, 4 p. m. ....	do.	0.1 c. c. sub-cutaneous.	102	
	May 4, 10 a. m. ....			104.8	Anthrax fever. Check dies to-day.
	May 5, 10 a. m. ....	2 c. c. sub-cutaneous.		102.5	
	May 5, 4 p. m. ....			101	
	May 6, 10 a. m. ....			102	
	May 7, 10 a. m. ....			102.5	
	May 8, 10 a. m. ....				Animal turned out in yard. Animal found dead. Post-mortem showed death from anthrax and cocci-diosis (lived 12 days).
	May 14, 10 a. m. ....				
Sheep 5.....	June 29, 10 a. m. ....	5 c. c. sub-cutaneous.			
	June 29, 12 m. ....	do.			
	June 29, 2 p. m. ....	do.			
	June 29, 4 p. m. ....	do.	0.1 c. c. sub-cutaneous.		
	June 30, 10 a. m. ....	do.			
	June 30, 12 m. ....	do.			
	June 30, 2 p. m. ....	do.			
	June 30, 4 p. m. ....	do.			
	July 2, 9 a. m. ....	do.		105.8	
	July 2, 10 a. m. ....	do.		104.3	
	July 2, 12 m. ....	do.		105.8	Died of anthrax at 2 p. m. on fourth day.
Sheep 6.....	June 29, 10 a. m. ....	do.			
	June 29, 12 m. ....	do.			
	June 29, 2 p. m. ....	do.			

TABLE 6.—*Experiments with precipitated anthraxase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxase.	Anthrax culture.		
Sheep 6 .....	1908.			° F.	
	June 29, 4 p. m....	5 c. c. subcutaneous.	0.1 c. c. subcutaneous.	.....	
	June 30, 10 a. m....	do.....	.....	.....	
	June 30, 12 m....	do.....	.....	.....	
	June 30, 2 p. m....	do.....	.....	.....	
	June 30, 4 p. m....	do.....	.....	.....	
	July 2, 8 a. m....	do.....	.....	102.2	Check guinea pig dies in 2 days.
	July 2, 10 a. m....	do.....	.....	102	
	July 2, 12 m....	do.....	.....	102	
	July 2, 3 p. m....	do.....	.....	102.8	
	July 2, 7 p. m....	do.....	.....	103	
	July 4, 10 a. m....	do.....	.....	.....	Animal dead of anthrax on sixth day.
Sheep 7.....	July 28, 10 a. m....	do.....	.....	.....	
	July 28, 12 m....	do.....	.....	.....	
	July 28, 2 p. m....	do.....	.....	.....	
	July 28, 4 p. m....	do.....	0.1 c. c. subcutaneous.	.....	
	July 29, 10 a. m....	do.....	.....	102.3	
	July 29, 12 m....	do.....	.....	102	
	July 29, 2 p. m....	do.....	.....	102.3	
	July 29, 4 p. m....	do.....	.....	102.6	
	July 30, 10 a. m....	do.....	.....	106	
	July 30, 12 m....	do.....	.....	106	
	July 30, 2 p. m....	do.....	.....	106	
	July 30, 4 p. m....	do.....	.....	106.2	
	July 31, 10 a. m....	.....	.....	.....	Found dead of anthrax on fourth day after inoculation of test dose.

## ANTHRAXOIN.

Anthraxoin was prepared and experimented with as follows: It consists essentially of a suspension, in carbolized normal salt solution, of dead, sporeless, anthrax bacilli. It was produced by inoculating, from a fresh anthrax carcass, bottles that had been filled completely with nutrient bouillon and plugged with hardest paraffin stoppers. In this way none but sporeless bacilli were introduced, and under the existing anaerobic conditions no spores could form. After all growth had ceased, at 35° C., these cultures were completely immersed in a water bath maintained at 55° C. for one hour, which was sufficient to devitalize the bacilli but not to destroy any antibodies they might contain. The dead bacilli were separated by filtration from the liquid in which they had grown, and those adhering to the Berkefeld filter were washed off in the requisite amount of carbolized normal salt solution. The suspension experimented with consisted of the bacilli that grew in 2,500 c. c. of bouillon, suspended in 50 c. c. of carbolized normal sodium chlorid solution. The details are given in Table 7.

TABLE 7.—*Experiments with anthraxoin.*

Animal.	Date.	Injections.		Temper- ature.	Result and remarks.
		Anthraxoin.	Anthrax cul- ture.		
Sheep 8.....	1908. Apr. 24, 10 a. m....	1 c. c. subcu- taneous.	.....	°F.	Sheep dies of anthrax in 2 days.
	May 8, 10 a. m....	2 c. c. subcu- taneous.	.....	.....	
	May 20, 10 a. m....	.....	0.25 c. c. sub- cutaneous.	.....	
	May 22, 10 a. m....	.....	.....	.....	
Sheep 9.....	Apr. 24, 10 a. m....	2 c. c. subcu- taneous.	.....	.....	Remains well.
	May 8, 10 a. m....	.....	0.25 c. c. sub- cutaneous.	.....	
	May 9, 10 a. m....	.....	.....	102.2	
	May 9, 4 p. m....	.....	.....	103	
	May 10, 10 a. m....	.....	.....	101.5	
Sheep 10.....	May 11, 10 a. m....	.....	.....	101.5	Sheep dies of anthrax in 3 days.
	Apr. 24, 10 a. m....	0.5 c. c. sub- cutaneous.	.....	.....	
	May 8, 10 a. m....	1 c. c. subcu- taneous.	.....	.....	
	May 20, 10 a. m....	.....	0.25 c. c. sub- cutaneous.	.....	
Sheep 11.....	May 11, 10 a. m....	1 c. c. subcu- taneous.	.....	.....	Sheep dies of anthrax in 2 days.
	May 24, 10 a. m....	.....	0.25 c. c. sub- cutaneous.	.....	
Sheep 12.....	May 11, 10 a. m....	1 c. c. subcu- taneous.	.....	.....	Sheep dies of anthrax in 3 days.
	May 20, 10 a. m....	.....	0.25 c. c. sub- cutaneous.	.....	
Sheep 13.....	May 11, 10 a. m....	1 c. c. subcu- taneous.	.....	.....	Sheep dead from anthrax in 2 days.
	May 23, 10 a. m....	5 c. c. subcu- taneous.	.....	.....	
	June 8, 10 a. m....	.....	0.25 c. c. sub- cutaneous.	.....	
	June 10, 10 a. m....	.....	.....	.....	
Sheep 14.....	May 11, 10 a. m....	1 c. c. subcu- taneous.	.....	.....	Sheep dead from anthrax in 4 days.
	May 23, 10 a. m....	5 c. c. subcu- taneous.	.....	.....	
	June 8, 10 a. m....	.....	0.25 c. c. sub- cutaneous.	.....	
	June 12, 10 a. m....	.....	.....	.....	
Sheep 15.....	Apr. 26, 9 a. m....	10 c. c. sub- cutaneous.	.....	103	Sheep dead from anthrax in 3 days.
	Apr. 26, 12 m....	.....do.....	.....	104	
	Apr. 26, 2 p. m....	.....do.....	.....	104	
	Apr. 26, 4 p. m....	.....do.....	0.25 c. c. sub- cutaneous.	104	
	Apr. 27, 9 a. m....	12 c. c. sub- cutaneous.	.....	103	
	Apr. 27, 12 m....	.....do.....	.....	103	
	Apr. 27, 2 p. m....	.....do.....	.....	103.5	
	Apr. 27, 4 p. m....	.....do.....	.....	103.5	
	Apr. 28, 9 a. m....	10 c. c. sub- cutaneous.	.....	104	
	Apr. 28, 2 p. m....	.....do.....	.....	103.5	
	Apr. 28, 4 p. m....	.....do.....	.....	104	
	Apr. 29, 10 a. m....	.....do.....	.....	.....	
Rabbit 32,.....	Mar. 8, 10 a. m....	0.5 c. c. sub- cutaneous.	.....	.....	
	Mar. 8, 1 p. m....	.....do.....	.....	104.1	
	Mar. 8, 4.30 p. m....	.....do.....	.....	105.4	
	Mar. 9, 9 a. m....	.....do.....	0.2 c. c. sub- cutaneous.	101.8	



TABLE 7.—*Experiments with anthraxoin*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxoin.	Anthrax culture		
Rabbit 32.....	1908. Mar. 10, 9 a. m.			°F. 105.8	Dead from anthrax in 2 days.
	Mar. 11, 9 a. m.				
Guinea pig.....	Mar. 8, 10 a. m.	0.5 c. c. sub-cutaneous.			Dead from anthrax in 2 days.
	Mar. 9, 10 a. m.		0.2 c. c. sub-cutaneous.		
	Mar. 11, 10 a. m.				
Rabbit 33.....	Mar. 16, 9 a. m.	0.1 c. c. sub-cutaneous.		101	Animal dead from anthrax in 2 days.
	Mar. 16, 11 a. m.			102.2	
	Mar. 16, 12 m.			102.5	
	Mar. 16, 4.30 p. m.			104.3	
	Mar. 17, 9 a. m.			101.5	
	Mar. 26, 9 a. m.	0.1 c. c. sub-cutaneous.		102	
	Mar. 26, 4 p. m.			103.5	
	Apr. 6, 9 a. m.		0.01 c. c. sub-cutaneous.		
	Apr. 8, 9 a. m.				
Rabbit 34.....	Mar. 16, 9 a. m.	0.2 c. c. sub-cutaneous.		102	Rabbit stupid.
	Mar. 16, 11 a. m.			103.6	
	Mar. 16, 12 m.			104.5	
	Mar. 16, 1.30 p. m.			105.3	
	Mar. 17, 9 a. m.			102	
	Mar. 26, 9 a. m.	0.2 c. c. sub-cutaneous.		102.5	
	Mar. 26, 2 p. m.			105	
	Apr. 6, 9 a. m.		0.01 c. c. sub-cutaneous.		
Rabbit 35.....	Apr. 8, 9 a. m.				Rabbit dead from anthrax in 2 days.
	Mar. 16, 9 a. m.	0.3 c. c. sub-cutaneous.		102	
	Mar. 16, 11 a. m.			103.2	
	Mar. 16, 12 m.			103.5	
	Mar. 16, 2 p. m.			104	
	Mar. 16, 4 p. m.			105	
	Mar. 17, 9 a. m.			102.5	
	Mar. 26, 9 a. m.	0.3 c. c. sub-cutaneous.		102	
	Mar. 26, 4 p. m.			104.5	
	Apr. 6, 9 a. m.		0.01 c. c. sub-cutaneous.		Dead from anthrax in 2 days.
	Apr. 6, 9 a. m.				

## A COMMERCIAL VACCINE IN PILL FORM.

A commercial vaccine which, according to the makers, consists of dead anthrax organisms in pill form was also tested. These small pills are placed under the skin by means of a trocar and are claimed by the makers to produce immunity to anthrax. Microscopic examination, as well as cultural and animal experiments, show that the claims of the makers, in so far as the vaccine being dead and harmless is concerned, are true. One can easily see with the microscope that these little pills consist of dead anthrax bacilli and their spores

held together in pill form by a proper excipient. The writer was unable, however, to verify the claim that they produce any immunity, as is shown in Table 2. A rabbit succumbed in six days, but as it is very rarely that a rabbit can be immunized by a vaccine, a sheep, which animal is easily protected, was also employed, with negative results.

TABLE 8.—*Experiments with commercial vaccine in pill form.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Vaccine.	Anthrax culture.		
Rabbit 36.....	1908. Apr. 7, 9 a. m.....	1 pill, subcutaneous.		° F.	
	Apr. 8, 9 a. m.....	1 pill (dissolved), subcutaneous.			
	Apr. 9, 4 p. m.....	do.....			
	do.....		0.01 c. c. subcutaneous.		
	Apr. 10, 4 p. m.....	1 pill, subcutaneous.			
	Apr. 11, 4 p. m.....			104	
	Apr. 13, 4 p. m.....			104.3	
	Apr. 15, 4 p. m.....				Dead from anthrax in 6 days.
Sheep 16.....	Oct. 17, 9 a. m.....	1 pill with trocar.			
	Nov. 2, 9 a. m.....		0.1 c. c. subcutaneous.		
	Nov. 4, 9 a. m.....				Sheep dead from anthrax in 48 hours.

## PREPARATION OF AN EFFECTIVE SINGLE VACCINE.

The single vaccine, like Pasteur vaccine, consists of cultures of attenuated anthrax bacilli, the only difference being in the degree of attenuation and that, as its name implies, it is applied only once, thus requiring a shorter time and only one handling of the animals.

To prepare such a vaccine or attenuated culture, the virulence of an already virulent culture of anthrax was exalted by passage through very young animals. A strain can in this way be produced which will kill a sucking rabbit in 24 hours. From the heart's blood of such a carcass tubes of bouillon are inoculated immediately after death. These cultures are then incubated at 42° to 43° C. for varying periods of time—from 12 to 18 days. On the twelfth day a subculture is made from one of the tubes and cultivated at 35° C. On the thirteenth and succeeding days subcultures are made from the remaining tubes until the series has been completed. These six subcultures of attenuated bacilli are now tested on guinea pigs and rabbits, and on sheep if possible.

More dependence is to be placed upon the animal test than upon the number of days the attenuation process is carried on. A culture of proper strength is generally obtained from tubes that have been attenuated for about 16 days. The proper culture will be one which will not kill rabbits, but which will kill a majority of guinea pigs in a delayed period, say 5 or 6 days. Such an animal should show no swelling at the point of inoculation, and the bacilli should be found only sparingly in the internal organs. When shaken in cultures such a vaccine shows a homogeneous clouding of the medium; no flocculi persisting, as occurs in virulent cultures.

As rabbits and guinea pigs can not be made immune to anthrax by vaccination of this kind, these animals are only useful in testing the pathogenesis of the cultures. For testing the immunizing property, sheep, which are easily immunized, were employed. Quite a number of sheep were used in the experiment to produce a safe, efficient single vaccine, and as regards the failures along this line, it is only necessary to say that they were entirely due to improper attenuation of the cultures. When the proper attenuation was reached there was no difficulty in immunizing animals by a single vaccination. This was effective against a subsequent, otherwise mortal dose of virulent anthrax bacilli, as the following experiments will show.

#### TESTS OF THE SINGLE VACCINE.

##### EXPERIMENT No. 1.

On April 15, 1909, a visibly pregnant cow was given subcutaneously 1 c. c. of single vaccine. She showed no ill effects whatever from the injection. On May 1 the animal was inoculated with 0.2 c. c. of virulent anthrax bacilli from a 24-hour culture which killed a check rabbit and a check cow in 48 hours. On May 19 the cow dropped a fully developed calf which when found was dead, lying with its head bent under the shoulder. Supposing that the calf had been asphyxiated, it and the membranes were buried. However, a platinum loop full of the discharge was plated and a pure culture of 60,000 anthrax bacilli was obtained. A guinea pig inoculated with a culture made from one of these colonies died of anthrax in 48 hours. The cow remained well, and subsequent daily cultivations from vaginal discharges showed no anthrax bacilli.

This experiment was extremely valuable, not only as showing that the cow had been immunized by a single vaccination, but also for showing not only that anthrax can be communicated not only to the fetus, but that it can be thus communicated by an immune mother. It also showed that anthrax bacilli may persist for at least 18 days in the body of an immune animal. When the cow was destroyed for various reasons on May 25 she was in perfect health, and cultures

and inoculations made with her blood showed no anthrax bacilli present. It is to be regretted that this animal could not have been kept for further observations and experiments.

#### EXPERIMENT No. 2.

On September 14, 1909, two sheep were vaccinated with single vaccine, each receiving 1 c. c., and showed no sickness therefrom. On September 28 each sheep was inoculated subcutaneously with 0.2 c. c. of a culture of anthrax bacilli whose virulence had been proven on a rabbit in the same experiment. The sheep at no time showed any sickness. On November 2 both sheep were again tested with virulent bacilli and showed no sickness.

#### EXPERIMENT No. 3.

On November 12, 1909, three sheep were vaccinated with 1 c. c. of single vaccine and showed no sickness therefrom. On November 27 one of the animals was tested with 0.2 c. c. of virulent bacilli, and on December 1 the other two were similarly tested. They at no time showed any sickness. These three sheep, together with the two used in experiment No. 2, were used later in experiments to produce an antibacterial serum.

#### A SERUM FOR PRODUCING IMMEDIATE IMMUNITY.

Although the favorable results from vaccination by the Pasteur system have been known for a long time, and owing to the cheapness of the vaccine it would seem that there is nothing more to be desired, the length of time required to produce immunity is one drawback in its use when one is endeavoring to check an existing outbreak of the disease. When vaccination is practiced a month in advance of the animals being turned out to pasture in the spring, the system of Pasteur vaccination is the proper one to use. In existing outbreaks, however, it is evident that any system that requires a month to become protective leaves much to be desired, as many animals could become infected and die before protection could be afforded them.

With the end in view of devising a method whereby an immediate immunity could be established in existing outbreaks or where an immunity could be brought about in a much shorter time than has been possible under the old system of vaccination, the writer has devoted a large portion of his time for the past three years. As it had been conclusively demonstrated that animals can be immunized by a single vaccine, thus cutting down one-half the period necessary for immunization by the old system of Pasteur, it was decided to experiment upon the production of an antianthrax serum by endeavoring to



hyperimmunize sheep after the manner of the production of anti-hog-cholera serum, with the exception that whereas in the latter work virulent blood was used, this was precluded in our experiments owing to the dangers attending the handling of large quantities of virulent anthrax blood. Instead, the blood used was that drawn from an animal immune to anthrax, and not from one sick with anthrax.

Briefly stated, the writer has produced an antibacterial serum by highly immunizing sheep through repeated inoculations, first of attenuated anthrax bacilli, and following these by inoculations of the most virulent races of the bacilli in increasing doses until the animal would withstand with impunity fifty thousand times the minimal lethal dose. Such a serum will protect a sheep against an otherwise mortal dose of bacilli and produce an immediate immunity. It is, therefore, a very valuable adjunct in working against the spread of the disease in existing outbreaks where the usual vaccination is being practiced. The serum will confer a passive immunity immediately, and thus protect the animal against fatal infection until the vaccine confers an active immunity. And should an animal which has been protected by the serum become infected with a virulent anthrax bacillus, the results of this infection will be the production of a much stronger immunity than the vaccine and serum would otherwise confer.

It is evident that if a single vaccine, or even a double vaccine, can be used in conjunction with an antibacterial serum to produce immediate passive immunity which will persist until the vaccine has had time to bring about active immunity a long step will have been made in controlling this formidable disease. The experiments with this end in view were carried out upon sheep as follows:

#### EXPERIMENTS WITH THE SERUM.

##### EXPERIMENT No. 1.

On December 15, 1909, a sheep immunized with single vaccine and a subsequent inoculation with virulent bacilli (see Experiments Nos. 2 and 3 with single vaccine) was bled from the carotid artery—the femoral artery being small and deep-seated—by means of a glass cannula and rubber tubing. When the blood had clotted, 500 c. c. of serum were decanted and injected into the inguinal regions of another sheep which had been similarly immunized. Microscopic examinations and inoculations into guinea pigs of this blood showed it to be free of anthrax bacilli. The injected sheep was lame in both hind legs the next day, but this lameness disappeared when the complete absorption of the injected blood had taken place, and no abscess formed. On January 12 this sheep was bled from the carotid artery,

and when the serum was collected it was preserved by adding to it one part of a 5 per cent solution of carbolic acid to each nine parts of serum. In making this addition of carbolic acid some coagulation will occur if the preservative and serum be not poured simultaneously into another vessel and stirred by a helper.

The above serum was without protective properties. All animals upon which it was used died of anthrax, as follows:

On January 13, 1910, 12 guinea pigs received increasing doses of serum from 0.1 up to 3 c. c. and simultaneously 0.1 c. c. of virulent anthrax culture. All of these animals died of anthrax in 48 hours.

On January 17, 1910, the amounts of serum were increased to 4 c. c., 5 c. c., 6 c. c., 7 c. c., 8 c. c., 9 c. c., and 10 c. c., with a simultaneous dose of 0.1 c. c. of virulent culture. The 7 animals thus tested also died in 48 hours.

On February 5, 1910, a sheep received subcutaneously 24 c. c. of the serum and 0.2 c. c. of virulent culture. A control rabbit died in 48 hours and the sheep died of anthrax in three days.

On February 9, 10, and 11 another sheep received daily 12 c. c. of the serum and on the 12th a test dose of virulent bacilli. This sheep lived four days and then died of anthrax.

These experiments demonstrated that the modified hog-cholera method of serum production could not be applied to anthrax, and it was then decided to try and bring about hyperimmunization by increasing doses of virulent bacilli repeated over a long period. The two sheep used in experiment No. 2 with single vaccine were employed for this purpose as follows:

#### EXPERIMENT No. 2.

On September 14, 1909, two sheep were vaccinated with 1 c. c. of single vaccine. On September 28 they were given a test dose of 0.2 c. c. of virulent bacilli, and remained well, while a check rabbit died in 48 hours. On November 2 each sheep again received 0.2 c. c. of virulent bacilli. On March 4, 1910, each sheep received 0.3 c. c. of virulent bacilli. On March 18 they received 1 c. c. of virulent bacilli, and on April 2 each sheep received 5 c. c. of virulent bacilli. A period of about seven months was thus consumed.

On April 14, 1910, these sheep were bled from the carotid artery, producing 1,900 c. c. of serum, to which was added one part of a 5 per cent solution of carbolic acid to each nine parts of serum as a preservative. Prior to the addition of the preservative tests for the presence of anthrax bacilli were made upon guinea pigs and by plate cultures. None were found.

This serum was used upon sheep in connection with virulent anthrax bacilli as shown in Table 9.

TABLE 9.—*Experiments with antianthrax serum.*

Animal.	Date.	Injections.		Results and remarks.
		Immune serum.	Virulent culture.	
Sheep 17.....	1910. Apr. 29	5 c. c. subcutaneous..	0.2 c. c. subcutaneous...	Sheep remains well. Rabbit dies in 48 hours.
Rabbit (control)	May 16	.....	.2 c. c. subcutaneous...	
	.....do.....	.....	.1 c. c. subcutaneous...	
Sheep 18.....	May 21	10 c. c. subcutaneous.	.1 c. c. subcutaneous...	Not sick. No bad result. Remained well. Died in 48 hours.
Rabbit (control)	June 20	.....do.....	.2 c. c. subcutaneous...	
	July 6	.....do.....	.5 c. c. subcutaneous...	
	May 21	.....	.1 c. c. virulent culture.	
Sheep 19.....	May 24	10 c. c. subcutaneous.	.1 c. c. subcutaneous...	Remained well.
Rabbit (control)	June 20	.....do.....	.1 c. c. subcutaneous...	
	July 6	.....do.....	.5 c. c. subcutaneous...	
	May 24	.....	.1 c. c. virulent culture.	Died in 48 hours.
Sheep 20.....	May 27	.....	.5 c. c. single vaccine...	Remained well.
	June 20	10 c. c. subcutaneous.	.1 c. c. virulent culture.	
	July 6	.....do.....	.5 c. c. virulent culture.	
Sheep 21.....	May 27	.....	.5 c. c. single vaccine...	Do.
Rabbit (control)	June 20	10 c. c. subcutaneous.	.1 c. c. virulent culture.	
	July 6	.....do.....	.5 c. c. virulent culture.	
	June 20	.....	.1 c. c. virulent culture.	Dead in 48 hours.
Sheep 22.....	June 1	5 c. c. subcutaneous.	1.0 c. c. single vaccine...	Remained well.
	June 18	10 c. c. subcutaneous.	.1 c. c. virulent culture.	
	July 6	.....do.....	.5 c. c. virulent culture.	
Sheep 23.....	June 1	5 c. c. subcutaneous.	1.0 c. c. single vaccine...	Do.
	June 18	10 c. c. subcutaneous.	.5 c. c. virulent culture.	
	July 6	.....do.....	.5 c. c. virulent culture.	
Sheep 24.....	June 1	5 c. c. subcutaneous.	1.0 c. c. single vaccine...	Do.
	June 18	10 c. c. subcutaneous.	.1 c. c. virulent culture.	
	July 6	.....do.....	.5 c. c. virulent culture.	
Sheep 25 (control).	June 18	.....	.1 c. c. virulent culture.	Died of anthrax in 48 hours (control for sheep 22, 23, and 24, for inoculations June 18).
Rabbit (control)	July 6	.....	.1 c. c. virulent culture.	Died of anthrax in 48 hours (control for sheep 22, 23, and 24, for inoculations on July 6).

No doubt the period of time occupied in hyperimmunizing the sheep which produced this highly protective serum can be very much lessened—probably one-half. It will be noted that the time covered was about seven months. This was not intentional, but as the sheep had already been immunized for another object, and not used, it was decided to carry out the idea of greatly increasing their immunity by the method followed. It will be seen by referring to the table that an idle period of about three months elapsed between November, 1909, and February, 1910, which might just as well have been employed for the purpose. It is also possible that by giving larger doses of virulent bacilli the immunizing property of the serum may be considerably increased.

That the degree of immunity can be established by the number of inoculations and the quantity of virulent culture used was clearly shown in sheep Nos. 18, 19, 20, 21, 22, 23, and 24. They had received

three injections of serum and three inoculations with bacilli, in very small doses, and while they themselves were immune (see Table 9) their blood, when tested after slaughter on July 18, did not protect other sheep.

It is also highly probable that sheep will produce a more effective antianthrax serum for their own species, and cattle for cattle, horses for horses, etc.; that is, the sera should be homologous. However, no effort was made to test this principle, as the great expense attending such experiments precluded their being carried out.

#### CONCLUSION.

The writer does not advise the abandonment of the old Pasteur system of vaccination against anthrax when it is practiced upon animals before they are turned out on the pastures in the spring of the year. When animals are dying, however, vaccination alone requires too long a period to protect, and it is in these outbreaks that the anti-anthrax serum should be used in conjunction with vaccine. The experiments have shown that a single vaccine may be used with good results. Where it is desired, however, the serum may be used simultaneously with the double vaccine or with a single vaccine.













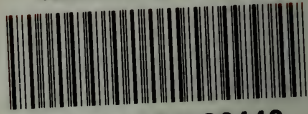








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